DOCTORAL THESIS

Energetics and life-history of olive baboons (Papio hamadryas anubis) in the Gashaka Gumti National Park

Lodge, Emily

Award date:
2012

Awarding institution:
University of Roehampton

Energetics and life-history of olive baboons (*Papio hamadryas anubis*) in the Gashaka Gumti National Park

by

Emily Lodge, MBiolSci

A thesis submitted in partial fulfillment of the requirements for the degree of PhD

Department of Life Sciences
University of Roehampton

2012

ABSTRACT

This thesis uses a number of novel methods to investigate how various measures of individual energetic status and condition vary within and between two troops of olive baboons (*Papio hamadryas anubis*) in Gashaka Gumti National Park, Nigeria. One troop is entirely wild-feeding whilst the other supplements its diet with crop-raiding, behaviour previously suggested to provide energetic benefits. Observations of activity budgets and feeding behaviour were combined with nutritional analyses of food samples to estimate energetic intake and expenditure amongst adult female baboons. Glucocorticoid (stress hormone), progesterone (reproductive hormone) and urinary C-peptide (an indicator of energetic status) levels of the same animals were assessed via analyses of faecal and urine samples. These data were used to investigate the effect of food-enhancement, between troops; the effect of reproductive state and rank, within troops; and the effect of variation in weather conditions and food availability across the nine month study period. Benefits of crop-raiding behaviour included elevated resting time, energy intake rates and reproductive success, and reduced feeding time and glucocorticoid levels in the crop-raiding troop as compared to the wild-feeding troop. Food-enhancement also appears to have buffered the crop-raiding troop's energetic status and stress levels against the effects of environmental stressors. Within troops, energy intake and expenditure rates varied between individuals in different reproductive states but not different ranks and neither factor significantly affected C-peptide or glucocorticoid levels. Rainfall had a considerable but variable influence on the baboons, being correlated with both positive and negative aspects of their behaviour and condition. Gashaka represents an extreme habitat for baboons, with high rainfall creating both a food and disease rich environment. The results of this study suggest that while low to moderate rainfall brings benefits, via increased food availability, heavy rainfall exerts a negative influence on the Gashaka baboons via increased disease risk.

ACKNOWLEDGEMENTS

Financial support from this project came primarily from the University of Roehampton, via a student bursary and a research grant from the department of Life Sciences. Additional funding was provided by the North of England Zoological Society, which also provides core funding to the Gashaka Primate Project, and the Leakey Trust.

Field work for this project was conducted with permission from the Nigerian National Parks Service and Professor Volker Sommer, director of the Gashaka Primate Project (GPP). I would like to thank the staff of GPP, in particular my excellent field assistants Halidu Ilyusun (Dan-Azumi), Maikanti Hassan, Bobbo Buba and Buba Bello, without whose expertise I would never have been able to find and keep up with the baboons. Thanks also go to Hammounde Guruza, Felix Vitalis, Buba Hammaselbe and Nodza George of GPP and my volunteer research assistants, James Tallant and Rosie Lodge.

I would like to thank my collaborator, Dr Sylvia Ortmann, and the technical staff at the Leibniz Institute for Zoo and Wildlife Research in Berlin. The nutritional analysis of the baboons' food items for this project was only possible with their expertise and assistance.

From the University of Roehampton, I would first like to thank my excellent supervisors, Dr Caroline Ross and Professor Ann MacLarnon, whose support and guidance throughout the last four years has made this thesis possible. I would also like to thank the technical staff, Amanda Morgan, Mary Mackenzie and especially Balbir Singh Josen, who provided so much help with my hormone analyses. Thanks also go to the staff and students of CREEA and CRE for providing such an inspirational and

enjoyable research environment. Special thanks go to Nienke Alberts, my fellow baboon researcher, who showed me the way to Gashaka, taught me how to live in the bush and generally provided support and friendship throughout my time in Nigeria. I also want to thank Charlotte Carne, Laura Buck, Laetitia Marechal, Astrid Willener and Stephanie Bird for all their comments and help with my work over the last year and for countless discussions about work-related, and less work-related, subjects.

Lastly I would like to thank my family. My husband, Tom Pollard, has supported me in so many ways since, and before, I started this project including following me to Nigeria and spending seven months living in a remote village, thereby making my time in Nigeria possible. My sister, Rosie, not only provided excellent research assistance to me during my field work but instilled in me a love of science and zoology from a young age and without whose inspiration I may never had embarked on this career path. Finally, my thanks go to my Mother and Father for everything they have given me, they made me the person I am today.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CONTENTS OF APPENDICES

Chapter 1

INTRODUCTION

This thesis investigates how various measures of individual energetic status or condition vary within and between two habituated troops of olive baboons (*Papio hamadyas anubis*) located in the Gashaka Gumti National Park, Nigeria. One troop is entirely wild-feeding whilst the other supplements its diet with anthropogenic foods raided from crop-fields within its range. Previous research on the two troops has identified differences in their activity budgets and reproductive success, which has been attributed to the energetic advantages of crop-raiding behaviour. The current study builds on this research by utilising non-invasive methods of assessing energetic status (calculated energy measures, urinary C-peptide levels), stress (faecal glucocorticoid metabolites) and reproductive condition (faecal progesterone metabolites) to compare the two troops.

In this introductory chapter a review of the relevant literature in the fields of primate feeding ecology and energetics is presented, followed by details of the study species and finally a presentation of the study's aims, objectives and hypotheses. The second chapter details the methods employed during the field, laboratory and statistical analysis phases of this project. In chapters 3-6 the results of four different areas of the project are presented: activity budgets and calculated energetic measures, urinary C-peptide as a measure of physiological energy balance, energetics and glucocorticoids (stress hormones) and progesterone and reproductive success. Each results chapter includes a discussion of its results with reference to the relevant literature and the results of previous chapters. In the final chapter the main findings from the discussion sections of the four results chapters are synthesised and overall conclusions are presented.

1.1. Primate diets

Primates, like all animals, require energy and protein for growth, reproduction and body maintenance, which they obtain from their food in the form of macronutrients: carbohydrates, protein and lipids. Primates also require smaller quantities of other nutrients, including macrominerals such as calcium and phosphorus, trace elements such as iron and copper, and vitamins (National Research Council, 2003; Lambert, 2007).

Most primates consume a wide variety of foods in order to obtain these nutrients, partly due to the fact that they are, predominantly, primary consumers, feeding far more on various parts of plants, which occupy the first trophic level, than on animal products. Foods from the first trophic level tend to demonstrate far more variability in their nutrient content than those from higher trophic levels. Leaves, especially young leaves, tend to be high in protein but low in easily available energy, whereas fruits tend to be high in energy but low in protein, and the nutritional content of flowers varies greatly between species. Because of this variability, most primary consumers, including many primates, must eat a variety of foods from different dietary categories each day in order to obtain the appropriate proportions of each essential nutrient (Milton, 1984).

Several factors influence the exact nutrient requirements of each primate species and the specific diets that individual animals adopt in order to satisfy these requirements. These factors include body size, digestive strategy, sex, age, reproductive state and interspecific competition.

1.1.1. Individual variation in nutritional requirements

Variation in energy and nutrient requirements exist within species such that individuals of different age, sex and reproductive state have different requirements. When males are larger than females, as in most sexually dimorphic nonhuman primates, energy requirements of adult males tend to be greater than those of adult females. Infants tend to require more energy per unit body mass than do adults. Protein requirements, relative to body size, also tend to decrease with age, as growth rate declines (National Research Council, 2003). However, as a percentage of energy, protein requirements increase with age (Oftedal, 1991). For female primates, pregnancy and lactation lead to increased energy and protein requirements. Pregnant women are recommended to increase their energy intake by 200-350 kcal per day, depending on the stage of pregnancy, and increases of 500 kcal per day are recommended during lactation (National Research Council, 2003). Increases in energy intake and time devoted to foraging during pregnancy, especially in the latter stages, and lactation have been observed in several species of non-human primates (Altmann, 1980; Lee, 1987; Silk, 1987; Dunbar and Dunbar, 1988; Muruthi *et al*., 1991; Srivastava, 1992). Pregnant and lactating animals are also known to decrease their energy expenditure, relative to cycling animals, in order to compensate for the extra energetic demands, in particular by increasing resting time relative to non-pregnant, non-lactating levels (Altmann, 1980; Rose, 1994; Barrett *et al.,* 2006). Protein requirements also increase during pregnancy and lactation (National Research Council, 2003). Pregnant yellow baboons have been shown to devote a greater proportion of their diet to protein rich seeds as their pregnancy progresses (Silk, 1987) and in humans, protein requirements increase by more than a third during lactation (National Research Council, 2003). Requirements for certain minerals also vary with age and reproductive status. For example, juveniles and lactating females require more calcium than other individuals (National Research

Council, 2003). Despite these observations, age and reproduction should be expected to have less of an effect on nutrient requirements in primates than in other mammals due to slow growth rates and low daily milk yields (Oftedal, 1991).

The differences in nutritional requirements between animals of different age, sex and reproductive state lead to differences in foraging behaviour. Amongst arboreal species, smaller individuals, which can include the females of some strongly sexually dimorphic species as well as juveniles, tend to spend a greater proportion of their time feeding on terminal branches and feeding higher in the canopy, than do larger individuals (Clutton-Brock, 1977). These differences are probably due to the fact that larger animals are not able to be supported high up in the canopy or on terminal branches and because of the lower risk of predation faced by larger animals. Females also tend to spend a greater proportion of their time feeding than males (Clutton-Brock, 1977; Rose, 1994). This is probably due to the combination of three factors, the fact that males do not incur the costs of pregnancy and lactation, the dominance of males over females present in most primate species, which results in better access by males to high quality feeding sites, and the fact that males tend to feed faster than females (Clutton-Brock, 1977; Rose, 1994). The exceptions to this rule are silverback male gorillas and male orangutans which, due to the extreme sexual dimorphism present in these species, need to consume substantially more food than females. Male primates tend to eat greater proportions of fruit and lesser proportions of protein rich insects and leaves in several species probably due to the greater relative protein requirements of females and juveniles (Clutton-Brock, 1977). Other differences include male orangutans eating less fruit and more bark than females and male chimpanzees eating more meat, probably due to the superior size and strength of the males (Clutton-Brock, 1977). In addition, males of several *Cercopithecus* species have been found to eat a greater proportion of fruit and smaller

proportion of leaves and insects than females, differences attributed to the elevated protein needs of females (due to pregnancy and lactation) and the fact that males may spend more time being vigilant for potential male competitors and so require easily accessible energy in the form of fruits (Gautier-Hion, 1980; Cords, 1986).

1.1.2. Chemical components of primate food

In order to satisfy its nutritional requirements, a primate must select which items to consume as food from those available in its habitat. Optimal foraging theory states that this choice should be based on maximising the amount of energy obtained per unit time spent searching for and handling those items (MacArthur and Pianka, 1966). Availability of food plays an important role in the food choice of primates and is likely to be particularly important in explaining variation in diet between populations of the same species (Clutton-Brock, 1977). Barton *et al.* (1993) found that plant part availability, as measured by its biomass, was the most important determinant of its contribution to olive baboon diets. However, this and other studies have found that other factors are also important in determining the contribution of various plant parts to primates' diets (Barton *et al.,* 1993; Chapman and Chapman, 2002). Although often omnivorous, primates are highly selective in the particular food items they consume, selecting a fraction of the potential food items available to them and ignoring or actively removing certain parts of otherwise desirable food items (Oftedal, 1991; Barton and Whiten, 1994). Energy content, occurrence of specific required nutrients, and presence of toxins or digestion inhibitors are all factors which can influence food choice in primates (Oftedal, 1991).

The energy that a primate food yields is supplied by the three macronutrients: carbohydrates, protein and lipids. A primate should therefore be expected to select food

items high in these three components. However, these macronutrients differ in a number of ways including the amount of energy they yield to the consumer so the preference of primates for each of them may vary (National Research Council, 2003).

Carbohydrates usually supply around 40 % of the energy needed by primates and make up 50-80% of the dry matter of leaves, fruit and seeds (National Research Council, 2003). They are present in primate foods in the form of soluble sugars, (monosaccharides and disaccharides) and complex sugars (polysaccharides). Soluble sugars provide primates with a rapidly available source of energy since monosaccharides can be absorbed directly and used as energy, without the need for digestion, and disaccharides need only to be hydrolyzed into monosaccharides before absorption (Lambert, 2007). Primates have a taste preference for soluble sugars, which allows them to select foods with a good source of easily available energy (Hladik and Simmen, 1998). The acuity of this taste preference is affected by a primate's foraging strategy. Primates which tend to make large energy investments in order to find scattered, high energy foods, such as ripe, fleshy fruits or insects, tend to have high test sensitivities for sugars and other soluble nutrients, whereas primates which make small energy investments to find more common, low energy foods, such as unripe fruit and leaves, have low taste sensitivity for soluble nutrients (Hladik and Simmen, 1998).

Polysaccharides can be further divided into two main types which differ in the ease with which they yield energy. Starch or starch-like polysaccharides are a plant's energy reserves and can be broken down readily by the digestive enzymes of primates and other vertebrates (Lambert, 2007). These types of carbohydrates are a rich source of energy and are present in large amounts in plant storage organs such as corms and bulbs, which are an important part of some primates' diets, particularly those species with little

access to food items containing large proportions of soluble sugars such as ripe, fleshy fruits (Barton and Whiten, 1994). The non-starch polysaccharides can also be divided into two types: soluble non-starch polysaccharides, which are not as easily digested as starch but can be broken down entirely by fermentation, and insoluble non-starch polysaccharides, or structural polysaccharides, usually referred to as fibre and generally very difficult to digest (Milton, 1984; Lambert, 2007).

The amount of energy in a food item which is available to an animal depends on the proportion and type of fibre in that food item (Oftedal, 1991). Some primates possess specific adaptations for the breakdown of hemicellulose, cellulose and lignin but others are unable to derive any substantial energetic or nutritive benefit from these cell wall components and, as a consequence, many species avoid foods containing high proportions of these components or have rapid gut transit times in order to remove this material as quickly as possible (Barton and Whiten, 1994; Chapman and Chapman, 2002). Small primates in particular tend to avoid food items containing large proportions of fibre, since they require a high rate of nutrient supply from their food (Milton, 1984)

The protein in a primate's diet provides it with both energy and essential amino acids. Amino acids are necessary for growth, reproduction and the replacement of tissues and nine of the 20 amino acids which primates require cannot be synthesised within the body. These so-called essential amino acids must, therefore, come from the diet (Lambert, 2007). When considering the effect of protein content on primate food choice, the amount of available energy and the specific amino acid content must be considered. For example, leaves usually contain high levels of essential amino acids but also often contain high levels of toxic and digestion inhibiting compounds, whereas

storage organs tend to contain fewer essential amino acids but also lower levels of toxic compounds. Thus, the availability of protein to a primate consumer tends to be restricted by limited digestibility in the case of leaves and lack of specific, required amino acids in the case of storage organs (Oftedal, 1991). In general, primates obtain most of their dietary protein from leaves, insects and other animal matter. Fruit tends to be low in protein and mature leaves tend to contain a lower proportion of protein than immature leaves. This is because, as leaves age, their structural components increase and protein is allocated to other plant parts such as seeds and storage organs (Lambert, 2007).

Primates have been shown to prefer foods containing high proportions of protein and specific, essential amino acids (Oftedal, 1991; Whiten *et al.,* 1991; Barton and Whiten, 1994; Chapman and Chapman, 2002). This can be achieved by primates selecting immature over mature leaves, by specifically selecting species which have leaves, fruit or seeds with high protein contents and by frugivorous primates supplementing their diets with leaves or insects (Oftedal, 1991). Primates are also able to select protein and mineral rich resources during critical periods of growth and reproduction. For example, young primates and pregnant or lactating females have been found to increase their intake of leaves and insects (Lambert, 2007).

Lipids are the third macronutrient and contain the highest concentration of energy, providing twice the energy of carbohydrates or protein per gram. As well as a source of energy, lipids are essential for specific functions associated with development and reproduction. Primates obtain most of their dietary lipids from insects, other animal matter, seeds and the arils of some plant-species (Lambert, 2007). In general little is known about primate consumption of lipids although there is evidence that suggests that

some food items are selected for their lipid content (Whiten *et al.*, 1991; Knot, 1998). For example, male orangutans have been shown to increase their calorific intake relative to females by including a particular fruit (*Neesia* sp.) in their diet, the seeds of which are 46% lipid. Females are generally unable to exploit this rich lipid source because of its large size and hard husk (Knott, 1998). However, the inclusion of lipids in the diet may be less important for primates than other mammals as the majority of primates live in warm, tropical environments where there is little need for insulating body fat. A study of the nutritional ecology of cercopthecine monkeys and chimpanzees in Uganda found that their lipid intake was generally low, although it did increase during times of high ripe fruit abundance which might suggest some preference for it (Conklin-Brittain *et al.*, 1998). Another study on the feeding behaviour of the fat-tailed dwarf lemur found that, unlike temperate hibernators, they did not increase their lipid consumption during their fattening period, prior to hibernation. The authors suggested that this was because the lemurs did not have to function at low body temperatures during hibernation since ambient temperatures rarely fell below 10^{o}C (Fietz and Ganzhorn, 1999).

Micronutrients (minerals and vitamins), unlike macronutrients, do not provide a primate with energy. However, they are essential for many bodily functions and their presence can influence primate food choice (Lambert, 2007). Minerals are inorganic elements which are involved in various physiological functions. They form part of biological molecules (e.g. iron in haemoglobin), are components in body fluids and activate various hormonal and enzymatic processes. Primates have been shown to select specific plant foods because of their mineral content (Lambert, 2007). For example, black and white colobus monkeys have been observed to travel long distances and tolerate the presence of other groups in order to feed from specific plants which are high in sodium and other minerals compared to their usual diet (Oates, 1978). The presence of specific

minerals has been suggested to be the reason for geophagy and for the ingestion of rarely consumed food items such as bark, petioles and caterpillars, since these items have been found to have high mineral contents in relation to commonly consumed primate foods such as fruit (Oates, 1978; Barton and Whiten, 1994; Lambert, 2007). Vitamins are organic compounds required in various physiological processes. Some vitamins, such as B and C, are water soluble which means they cannot be stored in the body and must be consumed daily, and others, such as A, D, E, K, are fat soluble which means they can be stored. There is little knowledge of primate vitamin requirements but, in general, the vitamin content of most primate foods is assumed to be more than sufficient to meet requirements (Lambert, 2007).

The energy a primate can obtain from a potential food item is influenced by the presence of toxic secondary compounds. These compounds are produced by plants in order to deter herbivores and act by interfering either directly with an animal's physiology or with the digestion of certain nutrients; these are toxins and digestion inhibitors respectively (Freeland and Janzen, 1974; Lambert, 2007). Toxins can lead to severe physiological damage and even death, and digestion inhibitors can severely reduce the availability of nutrients to primates, for example tannins, which are polyphenolic compounds, act by forming insoluble complexes with proteins (Lambert, 2007). A primate must therefore not only choose foods which maximise its energy and nutrient intake and minimise its fibre intake but also those which minimise its intake of secondary compounds. As expected, the presence of condensed tannins and other secondary compounds in plant material has been shown to be negatively related to feeding behaviour in several primate species (Barton and Whiten, 1994; Lambert, 2007).

The presence of secondary compounds varies between species and plant parts and between the same plant parts at different ages (Freeland and Janzen, 1974). For example, leaves tend to have higher levels of secondary compounds than other plant parts (Conklin-Brittain *et al.*, 1998) and the concentration of condensed tannins tend to decline in fruits as they age. This decrease occurs alongside an increase in sugar content and, presumably, prevents the fruit from being eaten before the seeds have fully developed (Hladik and Simmens, 1998).

The chemical components of a food item combine to give its dietary quality. Dietary quality will increase with increasing energy and protein and decrease with increasing levels of fibre and secondary compounds. Some studies have used the protein to fibre ratio of a food item as a measure of dietary quality and others have used the ratio of protein to fibre plus secondary compounds. Both measures have been shown to correlate well with selection of food items by primates (Barton *et al.*, 1993; Chapman and Chapman, 2002). Positive correlations have also been found between the protein to fibre ratio of mature leaves and the biomass of Asian (Waterman *et al.*, 1988) and African (Oates *et al.*, 1990) colobines and Madagascan lemurs (Ganzhorn, 1992). On a smaller scale, this index of food quality has been found to predict biomass of red colobus monkey groups in different areas of a single forest in Uganda (Chapman and Chapman, 2002). These patterns provide evidence for the importance of the protein to fibre ratio to primate food choice, since large numbers of primates can be supported by areas in which common food items have a large protein to fibre ratio. Which of these ratios is more appropriate depends on how effective a primate's secondary compound detoxification system is. Primates with very effective detoxification systems, such as colobine monkeys which can perform detoxification in the gut, may not show a significant tendency to avoid plant parts which contain high levels of secondary

compounds and so the protein to fibre ratio may well be sufficient to predict food choice in these species (Chapman and Chapman, 2002).

While some studies have emphasised the importance of protein to primate food choice, others have found no such effect (Oftedal, 1991; Dasilva, 1992). Oftedal (1991) suggested that protein deficiency is unlikely to be a problem for most primate species and it has also been suggested that studies which have focussed primarily on protein: fibre ratios would benefit from also considering total calorific content of selected food items (Dasilva, 1992).

Studies which aim to investigate the determinants of primate food choice will be interested in all the various factors which may correlate with the consumption of specific food items, in particular their phytochemical content. However, studies which aim to investigate how a primate's food intake affects its body condition, reproductive success and ultimately its fitness will gain more from focussing on factors that have been shown to impact on these features, such as energy intake.

1.2. Energy balance and fitness

Accurately determining lifetime fitness in long-lived animals such as primates is often impractical. Because of this, most studies of primate populations that aim to investigate the effect of factors such as food intake or rank on fitness, including those discussed below, use surrogates for life-time fitness, such as current physical fitness or body condition and various measures of reproductive success such as interbirth intervals (IBIs) and size of offspring (Altmann, 1998). The studies discussed below all provide some evidence for the link between energy balance and fitness. However, as with fitness, not all measure or discuss energy balance directly. Food intake, energy

expenditure, habitat quality and environmental conditions, amongst other factors, can be used as proxies for energy balance.

1.2.1. Energetics and reproduction

Considerable evidence for a link between energy balance and reproductive success has been supplied by human studies. Unfavourable energetic conditions have been linked to decreases in ovarian hormone levels and menstrual frequency and regularity, all of which are associated with decreased probability of conception (Ellison, 1990; Ellison, 2003). Unfavourable energetic conditions during pregnancy have also been linked to decreases in gestation length and birth weight, both of which are related to infant mortality (Ellison, 2003).

In a study of human female energetics and fertility in a rural, agricultural population in the Democratic Republic of Congo, Bentley *et al.* (1998) found that ovarian hormone concentrations were significantly lower than those of healthy Western controls and were also lower amongst women who lost weight during the study compared to those whose weight remained stable. Reduced weight and lower ovarian hormone levels during the 'hunger season' were also associated with a decrease in conceptions during this period which resulted in marked birth seasonality within the population (Bentley *et al.*, 1998). A similar effect on ovarian function has been found amongst Western women on weight loss diets and those who do significant amounts of aerobic exercise (Ellison, 1990). Although within population lowering of ovarian hormone levels has been convincingly linked to energetic stressors and reduced fertility, variation in ovarian hormone levels, between populations, due to differences in energy intake and expenditure, appears to be the norm and also to have little influence on fecundity (Vitzthum, 2009). For example, successful conception and implantation in rural Bolivian women are characterised by

substantially lower progesterone levels (50-70% depending on cycle stage) than for women from Chicago (Vitzthum *et al.*, 2004).

As well as the conception probability of each of her cycles, a female's life time reproductive success will be affected by the total duration of her reproductive period, which will be strongly influenced by her age at menarche, and her rate of reproduction, which will be strongly influenced by the length of her inter-birth intervals (IBIs). Length of lactational amenorrhea, which is one the major determinants of IBIs in human natural-fertility populations, has been linked to energetic condition with dietary supplementation shown to decrease the level of ovarian suppression during this period (Ellison, 1990; Ellison, 2003). Age at menarche has also been shown to vary with environmental conditions in humans, occurring later in girls exposed to greater nutritional stress or higher levels of physical activity, such as those experienced by rural populations in developing countries, than in girls from affluent, Western populations who face fewer energetic stressors (Bentley, 1999). Within Western populations, girls on weight control diets, and those who undertake large amounts of physical exercise, also experience menarche later relative to their peers (Bentley, 1999).

Similar patterns have been found by comparing captive and wild primates. Captive chimpanzees reach menarche three years earlier than wild chimpanzees, experience shorter periods of adolescent sub-fecundity, younger age at first birth, greater total fertility rates and shorter IBIs (Bentley, 1999). Captive baboons also have IBIs significantly shorter than those of their wild counterparts (Altmann and Altmann, 1977; Garcia *et al.*, 2006). These kinds of differences are generally attributed to the better nutrition and reduced energy expenditure experienced by captive animals (Bentley, 1999; Garcia *et al.*, 2006).

In wild chimpanzees, habitats containing high densities of preferred foods were associated with increased ovarian hormone production by females, shorter IBIs and higher infant survival (Emery Thompson *et al.*, 2007). In the same community, frequency of sexual swellings, concentration of oestrogen and waiting time to conception associated positively with consumption of energy rich, drupe fruits (Emery Thompson and Wrangham, 2008). In Bornean orangutans, periods of high fruit availability and positive energy balance (as measured by caloric intake and absence of urinary ketones) were associated with high levels of ovarian hormones compared to periods of low fruit availability (Knott, 1997). In baboons, energy intake during infancy was found to effect life-time reproductive success. In his seminal study of juvenile yellow baboons' foraging behaviour, Altmann (1998) found that differences in energy intake by 'yearling' females (aged 7-15 months [Altmann and Altmann, 1977]), relative to optimum levels, accounted for over 90% of the variance in female fecundity and over 80% of the variance in female reproductive success, defined as number of surviving juveniles. Studies of baboons have also demonstrated a link between adult body mass and reproductive performance (Bercovitch, 1987). For example, in a provisioned semi-free ranging group of baboons, heavier mothers conceived more quickly than lighter mothers following a birth and had shorter IBIs (Garcia *et al.*, 2006). Other measures of body condition have also been linked to reproductive success in non-human primates. For example, a study of free ranging, partially provisioned rhesus macaques found that greater body fat and muscle mass were both associated with increased fertility in females (Campbell and Gerald, 2004).

A link between energy intake and reproductive success is also suggested by the fact that both human and non-human primate females increase their food consumption, relative

to cycling females, when pregnant or lactating, as discussed above (Silk, 1987; National Research Council, 2003).

How energetic status modulates reproduction in humans

The link between unfavourable energetic conditions and reduced reproductive function is better studied in humans than in other primates, which is why only human studies are discussed in this section. Despite various factors which mark human reproductive function apart from that of other primates (Hrdy, 1999), I assume here that the mechanisms modulating the relationship between energetics and reproduction are similar to those acting in non-human primates. The mechanisms by which energetic condition affects reproductive output vary according to the stage of reproduction, including conception, gestation and lactation. The probability of conception and the successful initiation of pregnancy vary in response to maternal energy availability via suppression of ovarian function. This suppression acts along a graded continuum in response to the severity of the energetic stressor, with mild energetic stressors, such as moderate weight loss or exercise, triggering mild forms of ovarian suppression and severe energetic stressors, such as rapid or extreme weight loss, resulting in severe ovarian suppression to the point of amenorrhea (Ellison, 1990). The mildest form of ovarian suppression is luteal insufficiency whereby progesterone production by the corpus luteum is below optimal levels, reducing the probability of successful implantation and increasing the risk of early pregnancy failure. Suppression of follicular development is also one of the milder forms of ovarian suppression and can be associated with lowered estradiol levels leading to the production of ova with a reduced probability of fertilisation. If follicular suppression is profound enough this can lead to ovulatory failure, reducing the probability of conception during that cycle to zero.

Ovulatory failure can lead to oligomenorrhea (menstrual irregularity) and eventually amenorrhea, the most extreme form of ovarian suppression (Ellison, 1990).

While the establishment of pregnancy is extremely sensitive to maternal energetic conditions, the maintenance of pregnancy is much less so (Ellison, 2003). However, the amount of energy invested in a pregnancy is affected by maternal energetic conditions, with unfavourable energetic conditions associated with reduced birth weight and reduced gestation length (Ellison, 2003). Foetal glucose levels are highly dependent on maternal levels, which will be determined by the mother's energetic condition. If foetal glucose levels in late gestation are insufficient for the rapid growth (especially of the brain) associated with this period, the foetus may have to metabolise its own fat reserves, a process that can lead to early parturition as well as reduced birth weight (Ellison, 2003).

The primary effect of maternal energetic condition during lactation is on the length of lactational amenorrhea and therefore the length of the likely interbirth interval. The timing of resumption of cycling following parturition depends both on the infant's need for milk and the mother's energetic condition, since these factors determine the availability of the mother's metabolic energy to support a new pregnancy (Ellison, 2003). The resumption of ovarian function may be triggered by a rise in available metabolic energy above normal, cycling levels. In support of this idea, urinary C-peptide levels, which as a by product of insulin synthesis are indicative of energy balance, have been found to rise steadily throughout pregnancy from below normal levels after birth to above normal levels just before the resumption of cycling (Valeggia and Ellison, 2009).

1.2.2. Food-enhancement

Comparison of wild foraging and food-enhanced primate groups provides another means to investigate the link between energy balance and fitness. Beneficial effects of various methods of food-enhancement, including deliberate provisioning, crop-raiding and scavenging from refuse sites, have been shown for several species of non-human primate. These include improvements to individual condition, such as increases in body mass, subcutaneous fat deposits and growth rate (Mori, 1979; Eley *et al.,* 1989; Strum, 1991; Altmann *et al.,* 1993; Kemnitz *et al.*, 2002; Altman and Alberts, 2005; Pusey *et al.,* 2005) as well as decreases in parasite loads (Eley *et al.,* 1989 but see Weyher *et al.,* 2006) and reproductive benefits, including increased birth rate and infant survivorship, shorter IBIs and accelerated sexual maturation (Mori, 1979; Sugiyama and Oshawa, 1982; Mori *et al.*,1997). These benefits are a result of the higher concentration of provisioned or scavenged food relative to naturally occurring food sources as well as the fact that human foods tend to have higher concentrations of macronutrients and tend to be easier to digest and to contain fewer toxins than wild-foods (Forthman-Quick, 1986; Fa, 1991; Strum, 1991).

Food-enhancement can also lead to changes in activity budgets, due to changes in foraging efficiency. Food-enhanced groups tend to decrease time spent foraging and travelling, and to increase time spent resting and in social activities relative to wild foraging groups (Altmann and Muruthi, 1988; Eley *et al.,* 1989; Singh and Vinathe, 1990, Fa, 1991). Changes in dominance hierarchies, group size and foraging party size have also been observed (Wrangham, 1974; Power, 1986; Sugiyama and Oshawa, 1982; Brennan *et al.,* 1985). Food-enhancement can also have negative effects such as increased exposure to human and domestic animal diseases (Rolland *et al.,* 1985; Eley

et al., 1986; Hahn *et al.,* 2003) and has also been linked to increases in aggression (Wrangham, 1974; Brennan *et al.,* 1985; Forthman-Quick, 1986; Hill, 1999).

Though some forms of food-enhancement have been relatively well studied, little investigation has been done into food-enhancement as a result of crop-raiding by primates (Higham, 2006). Crop-raiding, which can include the theft of harvested crops from stores as well as of growing crops from fields, is a particular kind of food-enhancement which differs in respect to other kinds since it involves conflict between the raiding animals and a farmer (Warren, 2003; Higham, 2006). The effects of this form of food-enhancement on surrogates of fitness may, therefore, be less than with the other forms of food-enhancement (Higham *et al.,* 2009a).

Crop-raiding animals face various risk factors unlikely to be found with other forms of food-enhancement such as risk of poisoning, snaring, trapping and hunting by farmers, as well as attacks by dogs (Higham, 2006). As with other forms of food-enhancement crop-raiding may be expected to increase aggression and stress amongst raiding animals (Wrangham, 1974; Fa, 1991). In fact, due to the presence of human-wildlife conflict, this form of food-enhancement might be expected to have a greater effect on the raiding animals' stress levels (Higham, 2006). Crop-raiding behaviour may even have a negative effect on reproduction if the physiological stress levels of cycling or pregnant females are raised high enough. In humans, high levels of physiological stress have been linked to decreased ovarian function and decreased birth weight of infants (Ledermar *et al.,* 1981; Matteo, 1987; Marcus *et al.*, 2001; Nepomnaschy *et al*., 2004). The benefits accrued from food-enhancement may be expected to vary more between group members, with age, sex and rank, when enhancement is from crop-raiding than when food is deliberately provisioned or even when it is scavenged from refuse sites

due to variation in willingness and ability to raid and the specific risk factors associated with crop-raiding. For example, Warren (2003) found that male baboons at Gashaka were more likely to crop-raid than females, and that adults were more likely to crop-raid than juveniles.

1.2.3. Dominance Rank

Another factor which can have a substantial effect on a female primate's body condition, reproductive success and ultimately her fitness, is her position in a dominance hierarchy. Dominance rank correlates significantly with body condition in female toque macaques (Dittus, 1998), and Hanuman langurs (Koenig, 2000) and with body fat in rhesus macaques (Small, 1981). Similarly, in chimpanzees more dominant females tend to be heavier and have a more stable body mass than subordinate females (Pusey *et al.,* 2005). Although little evidence has been found for a link between physical condition and rank in baboons (Silk *et al.,* 2005) associations have been found between rank and various measures of reproductive success. For example, higher ranking female baboons have shorter IBIs and produce more offspring with a better chance of survival, which tend to grow faster, be heavier as juveniles and, in the case of females, reach menarche earlier than those of lower ranking females (Bulger and Hamilton, 1987; Altmann *et al.,* 1988; Johnson, 2003; Altmann and Alberts, 2005; Garcia *et al.,* 2006 but see Packer, 1995 for a negative association between rank and reproductive function). Reproductive advantages of high rank have also been found in rhesus macaques (Drickamer, 1974) vervet monkeys (Whitten, 1983) Hanuman langurs (Borries et al., 1991) and long tailed macaques (van Noordwijk and van Schaik, 1999).

One way in which rank can affect body condition and reproductive success is via differences in food intake (Barton and Whiten, 1993; Cheney *et al.*, 2004). High ranking

white-faced capuchins experienced higher energy intake rates during feeding than lower ranking individuals (Vogel, 2005). Similarly dominance rank correlated positively with net energy gain in Hanuman langurs (Koenig, 2000). In the case of baboons, higher ranking females have higher rates of nutrient acquisition than lower ranking females (Barton and Whiten, 1993), experience fewer interruptions during feeding bouts (Post *et al.,* 1980), exclude lower ranking females from high quality, arboreal feeding sites (Barton, 1993) and are generally thought to have access to higher quality foods (Altmann and Alberts, 2005).

Social rank can only affect a female primate's fitness if a female dominance hierarchy exists in her social group, and although these types of hierarchies are the norm in most cercopithecine species they are not ubiquitous (Barton and Whiten, 1993). The likelihood of a primate group having a female dominance hierarchy depends on the distribution of the main food sources and predation risk (Barton and Whiten, 1993; Barton *et al.,* 1996). Where food sources are clumped, and therefore defendable, strong between group competition for food will exist which will favour the formation of female coalitions. This in turn will favour female philopatry and the formation of female dominance hierarchies. In contrast, when food is evenly distributed, and hence not defendable, female dominance hierarchies are unlikely to occur (Wrangham, 1980; Barton *et al.,* 1996; Sterck *et al.,* 1997). High predation pressure will increase within group feeding competition, since it will favour the formation of large, cohesive groups and will therefore increase the likelihood of dominance hierarchy formation. Low predation pressure will decrease the chances of dominance hierarchies, even in the presence of clumped resources, since members of the group will be able to forage individually or in small groups due to the decreased predation risk (Barton *et al.,* 1996).

Even when a female dominance hierarchy is present the likelihood that rank will have an effect on the food intake and body mass of individuals will be influenced by food distribution (Vogel, 2005; Garcia *et al.,* 2006). Garcia *et al.* (2006) found no effect of rank on food intake in groups of provisioned, semi-free ranging olive baboons, and suggested that this was because dominant females were unable to monopolise the evenly distributed food that the baboons were supplied with. Despite no link between rank and body condition in this population higher ranking females did have shorter IBIs than lower ranking females (Garcia *et al.*, 2006). This study highlights the fact that not all rank related differences in reproductive success are governed by differences in food intake.

1.2.4. Urinary C-peptide as a physiological measure of energetic status

A relatively new method by which energy balance can be related to fitness and its surrogates is the analysis of serum and urinary C-peptide levels. C-peptides are produced during the synthesis of insulin and in equal quantities. However, unlike insulin, C-peptides are not quickly broken down and so their concentration in the blood or in urine can be used to monitor the rate of insulin production (Deschner *et al.,* 2008). Since insulin regulates the synthesis and utilisation of energy reserves, C-peptide concentration can provide a sensitive indicator of energy balance. A high C-peptide concentration indicates a high insulin concentration, which is associated with a positive energy balance (energy intake exceeds output) whereas a low C-peptide concentration suggests a negative energy balance (energy output exceeds input) (Sherry and Ellison, 2007).

The link between energy balance and fitness is supported by the following examples. In humans, C-peptide levels have been shown to correlate positively with body mass index

(Polonsky *et al.*, 1988) and with calorific intake in recovering anorexic patients (Yoshida *et al.*, 2006). In chimpanzees, urinary C-peptide levels are higher in captive than in wild animals and, in the wild, C-peptide levels are higher during periods of high fruit abundance compared to periods of low fruit abundance (Sherry and Ellison, 2007). In wild Bornean orangutans, urinary C-peptide levels correlate significantly with food availability and energy intake (Emery Thompson and Knott, 2008) and in a study of captive bonobos increases in urinary C-peptide levels were significantly correlated with increases in body mass during a period of re-feeding following food restriction (Deschner *et al.*, 2008).

1.2.5. Glucocorticoids as a physiological measure of stress

Measurement of glucocorticoid metabolites in faecal or urine samples provides another non-invasive method for quantifying a wild primate's condition. Glucocorticoids are produced in response to stressful stimuli and act to redirect energy away from non-essential functions towards mitigating the immediate effect of the stressor (Sapolsky *et al.*, 2000). At baseline levels, glucocorticoids also act to regulate energy acquisition, deposition and mobilisation (Busch and Hayward, 2009). For these reasons, glucocorticoid metabolites have been used frequently in primatological research in order to assess the condition of individual animals in regards to exposure to energetic or psychosocial stressors (e.g. van Schaik *et al.*, 1991; Beehner *et al.*, 2005; Emery Thompson *et al.*, 2010).

1.3. Study species and site

1.3.1. Baboon studies

Baboons (*Papio hamadryas*) are commonly considered to consist of five subspecies (olive baboons, *P. h. anubis*; yellow baboons, *P. h. cynocephalus*; chacma baboons, *P.*

h. ursinus; Guinea baboons *P. h. papio* and hamadryas baboons, *P. h. hamadryas*), although this taxonomic classification is debated (e.g. Zinner *et al.,* 2011). However they are classified, baboons are considered to be one of the most ecologically and geographically diverse groups of non-human primates, occurring throughout most of sub-Saharan Africa and, arguably, occupying a wider variety of habitats than any other non-human primate (Altmann and Alberts, 2003a; Henzi and Barrett, 2003). Baboons are also one of the most intensively studied non-human primates and detailed studies of baboon behavioural ecology, many long-term, have been carried out at a large number of sites throughout sub-Saharan Africa (Dunbar, 1992). Alongside the ecological variation is a considerable amount of variation in diet, life-history traits and social systems which has lead to a large number of investigations into the links between these factors (e.g. Dunbar, 1992; Barton *et al.*, 1996; Hill *et al.*, 2000; Hill and Dunbar, 2002).

Baboons are selective omnivores and will eat a very wide range of foods including seeds, roots, leaves, fruits and flowers from plants as well as invertebrates and small amounts of vertebrate prey when available (Kummer, 1971; Whiten *et al*., 1991) As the environment occupied by the different baboon subspecies varies so does their diet. However, baboon diet varies as much within subspecies, between populations and throughout time, as it does between subspecies (Altman, 1974). The social behaviour of baboons is affected by the distribution of resources in the environment it lives in (Barton *et al.,* 1996; Strier, 2003). High quality food items, such as ripe fruit, tend to occur in defendable, discrete patches, whereas low quality food items such as grass tend to be distributed evenly throughout the environment (Strier, 2003). If a population of baboons inhabits an area rich in patchily distributed, high quality food items, individuals, especially females, should be expected to form coalitions with other,

preferably related, individuals in order to defend resources against conspecifics. This in turn will favour female philopatry, since both direct and inclusive fitness benefits can be accrued from forming coalitions with kin, and the formation of female bonded groups, as is the case for the olive baboons at Laikipia, Kenya (Barton *et al.,* 1996). In contrast, if a population inhabits a poor quality habitat with evenly and sparsely distributed resources female-female coalitions and bonding will not be strongly selected for. This pattern has been found amongst the chacma baboons at Drakensberg, South Africa (Barton *et al.,* 1996). Habitat quality also affects population density and therefore group size since the area required to support an individual baboon increases as habitat quality declines. For example one baboon may require 39 hectares of savannah or semi-desert habitat to support it whereas just three hectares of forest habitat may provide equivalent resources (Dunbar, 1988).

As well as the lack of distinction between the five subspecies in terms of diet, the basic biology and body size also show little variation (Dunbar, 1988). Like most primates, baboons have slow life histories compared to other mammals of similar size, not reaching full sexual maturity until they are around five years old, in the case of females, and eight years in the case of males. All subspecies are highly sexually dimorphic with adult males typically weighing twice as much as adult females. Where subspecies do vary substantially is in their social system, in terms of group size, social structure and mating system. What is less clear is whether these differences reflect genuine genetic variation, which came about due to differences in past selective pressures, or plasticity of social behaviour in response to differences in current ecological conditions (Dunbar, 1988; Henzi and Barrett, 2003).

Despite the wide geographical spread of baboons across sub-Saharan Africa, the vast majority of studies have focussed on southern and eastern populations with western populations being almost entirely neglected until recent years (Higham *et al.,* 2006; Kunz and Lisenmair, 2008). In fact just three of the 31 study sites which provided data for Dunbar's (1992) review were situated in West Africa. Since this review, detailed studies of baboon ecology and behaviour have been carried out at just two additional sites in West Africa, one in the Ivory Coast (Kunz and Linsenmair, 2008) and the other in Gashaka Gumti National Park (GGNP) in Nigeria (e.g. Ross *et al.,* 2011; Warren *et al.,* 2011). The geographical bias in baboon study sites is particularly noteworthy due to the fact that the Western regions of the baboons' range differ substantially from the better studied eastern and southern regions in several important respects. West and East Africa occupy distinct phytogeographic regions with significantly higher above ground forest biomass per unit area in West Africa and differences in dominant vegetation type between the regions (Kunz and Lisenmair, 2008). The majority of eastern and southern baboon study sites are dominated by Acacia wooded savannah or savannah grassland whereas much of sub-Saharan West Africa is dominated by a species rich forest-savannah mosaic habitat (Kunz and Lisenmair, 2008). Another major difference between the two regions is that West Africa has a far lower density of large predators than East Africa (Kunz and Lisenmair 2008). Previous studies of Western baboon populations have also suggested significant differences in the behaviour and structure of eastern and western populations with a tendency in Western populations towards lower individual densities, smaller group sizes, and increased frugivory (Warren, 2003; Kunz and Lisenmair, 2008).

1.4. Study aims

The overarching aim of this study is to investigate how energy balance influences reproductive success in baboons, in particular, by examining how energy intake and expenditure relate to physiological measures of energy balance, stress and reproductive condition and how these factors vary within and between two baboon troops within a population.

1.4.1. Specific objectives

The study focuses on the adult female members of two previously habituated baboon troops within the Gashaka region of the Gashaka Gumti National Park, Nigeria. One troop, Kwano troop, is entirely wild-feeding whilst the other, Gamgam troop, supplements its diet with anthropogenic foods raided from crop-fields within its range, a behaviour that is predicted to offer energetic benefits. The following is a list of the study's specific objectives:

- To estimate the **energy balance** of individual female baboons in the Kwano and Gamgam troops over nine months, by measuring activity budgets (to estimate energy expenditure) and food intake (to estimate energy intake).
- To obtain physiological measurements of the baboons' physiological **energetic status** from urine samples through analysis of C-peptides.
- To assess the baboons' physiological **stress levels** through analysis of glucocorticoid levels in faecal samples
- To assess the baboons' **reproductive condition** through analysis of progesterone levels in faecal samples.
- To investigate the relationships between energy balance and the physiological measures of energetic status, stress and reproductive condition and analyse these

measures in relation to previously collected data on the birth and infant mortality rates of the two troops.

1.4.2. Hypotheses

The data collected for this study will be used to test three main hypotheses each referring to the condition of the baboons. The term 'condition' is used here as an umbrella term encompassing all aspects of the baboons' health, energetic status and well-being. The measures of condition used in the current study are calculated energy balance, physiological energy balance (urinary C-peptides), stress hormone (glucocorticoid) levels and reproductive hormone (progesterone) levels. The assumption is that an animal in better condition will have higher energy balance, lower stress levels and higher reproductive hormone levels. The three hypotheses relate respectively to between troop, within troop and temporal variation in the baboons' condition:

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits which translate to better condition relative to Kwano troop.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary.

Hypothesis 3: The baboons' condition will vary throughout the year in response to variation in weather patterns and food availability which affect the baboons' energetic costs and the amount of energy available to them in the environment.

Specific predictions relating to each of the condition measures are included in the appropriate results chapters.

Chapter 2

FIELD AND LABORATORY METHODS

Field work took place at Gashaka Gumti National park, Nigeria, between February and December 2009 and consisted of behavioural observations of the adult female members of two habituated, wild olive baboon troops as well as the collection of faecal, urine and plant food samples. The first month of field work (February 2009) was spent learning how to track and identify the individual members of both baboon troops and in trying out and finalising the methods to be used. This pilot work was followed by two field work seasons, March-June and August-December 2009, during which time 32 weeks of data were collected.

2.1. Field site and study troops

Field work took place in the southern section of Gashaka-Gumti National Park. This National Park, situated in the east of Nigeria on the Cameroonian boarder, is the largest in country and is located between $6^{o}55'$-$8^{o}05'$N and $11^{o}11'$-$12^{o}13'$E (Dunn, 1998). The park encompasses a wide variety of habitat types from grassland and montane forest, including the highest peaks in Nigeria, to lowland forests, woodland savannah and swamps. The northern, Gumti, region of the park, consists of scrub and northern Guinea savannah while the southern, Gashaka, region of the park is generally more mountainous and consists of a mosaic of vegetation types including southern Guinea savannah woodland, which is denser than the northern type, and lowland and gallery forest (Sommer and Ross, 2011).

This study is part of a larger, long-term research and conservation project based in Gashaka-Gumti National Park, the Gashaka Primate Project (GPP), run by researchers

at University College London and the University of Roehampton since 2000. One of GPP's main focuses has been the comparison of two troops of olive baboons, habituated by Ymke Warren in 2000, one which feeds only on wild-foods and another which supplements its diet of wild-food with crop-raiding (e.g. Ross *et al.*, 2011; Warren *et al.*, 2011). The range of the crop-raiding troop, Gamgam troop, is situated just outside the border of the National Park close to the village of Gashaka, and the troop frequently engages in raids upon the agricultural fields belonging to the Gashaka villagers, along the banks of the river Gamgam (Warren, 2003). The range of the non-crop-raiding troop, Kwano troop, occupies a relatively undisturbed area approximately 10km away from Gashaka village. The Kwano range is situated at a higher altitude and contains a higher proportion of lowland and gallery forest and a lower proportion of Guinea savannah than the Gamgam range. The climate at the two sites is similar but Kwano tends to experience slightly lower temperatures and higher rainfall and humidity than Gamgam (Warren, 2003; section 2.1.2). In accordance with results of previous primate studies (described in section 1.2.2.), the food-enhanced, crop-raiding troop demonstrates significant reproductive advantages over the entirely wild-feeding troop both in terms of decreased IBIs and lower infant mortality (Higham *et al.*, 2009a).

As discussed in section 1.3.1, despite the large number of baboon studies, few have taken place in West Africa (Dunbar, 1992). The GPP site is significant not only for its geographical position in West Africa but also because it is situated close to the southern limits of the range of baboons in this region. It also has the highest annual rainfall of all the baboon study sites, by around 500mm, and one of the highest temperatures (Higham *et al.*, 2009a). The habitat structure, including large areas of closed forest alongside Guinea savannah mosaic habitat, is also unusual for baboons (Higham *et al.*, 2009a). For these reasons, the results of investigations from this site may provide insights into

what climatic or ecological characteristics restrict the distribution of baboons, as well as providing additional information which could be used to test or improve ecological explanations and models for the variation in baboon behaviour and social structure in different populations.

2.1.1. Troop composition throughout study period

Throughout the study period, Gamgam troop had between 20 and 22 members (table 2.1). One adult male (FEL) disappeared in the 2nd month of the study (last sighting 12/04/09), one new adult male (DWN) was observed associating with the troop right at the beginning of the study period (first sited 15/02/09) and became more closely affiliated with the troop as time passed. Another addition to Gamgam troop was the birth of a female infant (DIA) in September 2009.

Table 2.1. Composition of two study troops throughout study period.

Demographic class	Age in years of demographic class [1]	Kwano		Gamgam	
		Mode	Range	Mode	Range
Adult Female	6-8 +	10	10	5	5
Adult Male	10 +	8	5-8	1	1-2
Subadult Female	4-6	2	1-2	0	0
Subadult Male	6-10	1	1	2	2
Juvenile Female	2-4	6	4-6	2	2
Juvenile Male	2-6	5	5-6	7	7
Infant Female	0-2	2	2-3	1	1-2
Infant Male	0-2	3	2-3	2	2
Total troop size:		37	30-38	20	20-22

1. Age/sex class definitions after Warren 2003.

The membership of Kwano group varied between 30 and 38 individuals. The variation in troop size and demography (table 2.1) took the form of two additions, the birth of two female infants (DRS and DEB) in August 2009, the transition of one female (BNI) and one male (BRY) infant to the category of juvenile, in June 2009, and the disappearances/ deaths of seven troop members: three adult males (DOG and DMN in June and DRO in August), one subadult female (KRM), one juvenile male (BRY), one

female infant (CFT), who all disappeared in September 2009, and a male infant (DNA) who disappeared in November 2009.

The 15 adult female members of Kwano and Gamgam troops, as well as one sub-adult female from Kwano troop (KRM), acted as the focal animals for the current project (table 2.2.). KRM was included as a focal animal for the whole of the study period since, although as a 6 year old, nulliparous female she was classed as a subadult at the start of the study period, she became pregnant for the first time during the study period (May 2009) and was therefore categorised as an adult female until she disappeared in September 2009.

Table 2.2. Focal animal IDs and information on offspring and reproductive state during study period

ID	Age/Sex class	Known offspring in troop during study	Reproductive state during 9 study months
Kwano focal animals			
BRA	af	JMU (jm), BRY_d (im)	6 months L, 3 months AC
DRK	af	BNI (i-jf)	3 months C, 6 months P
FDI	af	ANN (jf), DND (im)	9 months L
KRM_d	saf/af	None	2 months C, 3 months P
KYE	af	FAR (jm), DRS (if)	5 months P, 4 months L
LDI	af	KRM_d (saf), BAK (jm), ADY (jm), DEB (if)	5 months P, 4 months L
LMI	af	NAW (jm), BTH (i-jf)	9 months C
MOM	af	AMY (jf)	2 months C, 6 months P, 1 month AC
SDY	af	TAL (jf), DJI (im)	8 months L, 1 months C
TOJ	af	RAB (saf), CFT_d(if)	5 months L, 4 months AC
YMK	af	MUR (jf), DNA_d (im)	7 months L, 2 months C
Gamgam focal animals			
BUW	af	PET (jm), FDI (jm), BIL (im)	9 months C
KAN	af	KOF (jm), DIA (if)	5 months P, 4 months L
MMK	af	KAN (af), GLO (jf), LEO (jm)	9 months C
MMW	af	AGU (sam), DOS (if)	9 months L
STR	af	SAB (sam), FRI (jm), JAN (jf), DEL (im)	5 months L, 4 months C

a=Adult, sa=Sub-adult, j=Juvenile, i=infant, f=Female, m=Male, C=Cycling, P=Pregnant, L=Lactating, AC= Annovulatory cycling (period between death of suckling infant and resumption of sexual swelling) $_d$=Disappeared/ died during study

2.1.2. Weather patterns and fruit availability throughout the year

Weather data

Weather data were collected at both field sites throughout the study period by the PhD and Masters' students studying at the sites, volunteer research assistants and the local field assistants. Minimum and maximum temperature, total daily rainfall and current humidity were recorded each day at 19:00. Any days for which data were missing (total number of missing days=37, median duration of missing data=2 days, max duration of missing data=7 days) were assigned the mean of the closest surrounding days i.e. the day before and the day after. Details of the weather patterns at both sites throughout the year of this study are given in table 2.3 and figure 2.1. There was no significant difference between the Kwano and Gamgam sites in terms of mean monthly minimum temperature (paired t-test, t=-0.745, d.f.=11, p=0.472), mean monthly maximum temperature (paired t-test, t=-0.237, d.f.=11, p=0.817), total monthly rainfall (paired t-test, t=0.879, d.f.=11, p=0.398) or mean percentage humidity at 19:00 (paired t-test, t=-1.1270, d.f.=11, p=0.284).

Table 2.3. Rainfall and temperature measures at the two field sites during the study period (1st January-31st December 2009).

Variable	**Gamgam**	**Kwano**
Total annual rainfall	1893 mm	2053 mm
Absolute minimum temperature	15^{o}C	15^{o}C
Absolute maximum temperature	41 ^{o}C	39 ^{o}C
Mean temperature	26.8 ± 0.27 ^{o}C	26.5 ± 0.24 ^{o}C

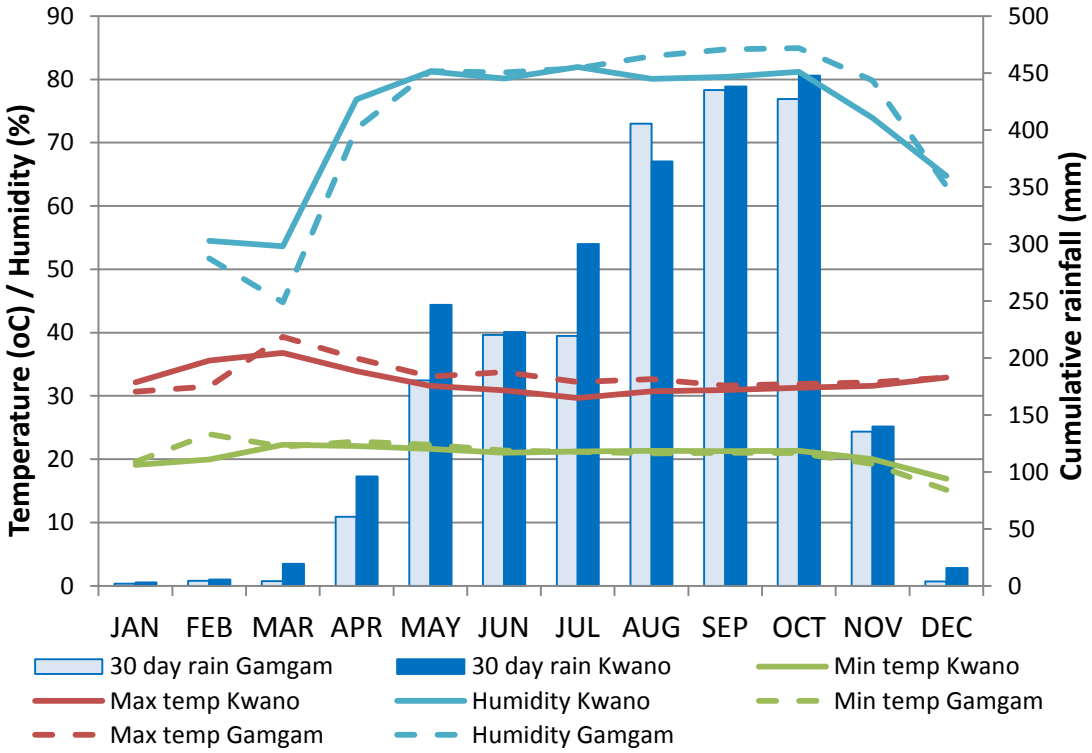

Figure 2.1. Variation in mean monthly values of cumulative rainfall over the previous 30 days (30-day rainfall) temperature and humidity at the two field sites throughout 2009.

Data used in all further analyses are daily minimum and maximum temperature and rainfall, the monthly mean minimum and maximum temperature and 30-day rainfall, which is the total rainfall over the past 30 days. This measure was used in preference to total monthly rainfall since the current ecological circumstances, and the baboons' behaviour, are more likely to be related to the amount of rainfall that had fallen in the previous 30 days than to a total monthly value which would include rainfall that occurred after the event of interest. Humidity values were not used in further analyses due to their close correlation with both daily and 30-day rainfall (Spearman's rank correlations, n=292 (1st March-17th Dec 09), Gashaka: daily humidity vs. daily rainfall: r_s=0.559, p<0.001; daily humidity vs. 30-day rainfall: r_s=0.683, p<0.001; Kwano: n=292, daily humidity vs. daily rainfall: r_s=0.591, p<0.001; daily humidity vs. 30-day rainfall: r_s=0.534, p<0.001).

As in many tropical regions, the Gashaka field site experiences two distinct climatic seasons, the wet season and the dry season. Sommer *et al*. (2004) defined the Wet season at the Gashaka field site as the period between mid-April and mid-October and the Dry season was defined as the period between mid-October and mid-April.

The data collection timetable (March-June and August-December 2009) was chosen because it allowed for data collection to take place throughout both the dry and wet seasons, including the transitions between the seasons. Data were collected during 14 dry season weeks (between 9/03 – 14/04 and 15/10 – 17/12) and 18 wet season weeks (between 15/04 and 14/10).

Fruit availability indices

The variation in fruit availability throughout the year has been assessed twice a month within the Kwano troop's home range since April 2002 (Sommer *et al.,* 2011). Experienced field assistants walked 8km transects, along which 1000 trees of various species were marked, and recorded the presence or absence of fruit in these trees and any associated vines. Three fruit availability indices were calculated using these data: a tree-fruit index, calculated as the sum of the Diameters at Breast Height (DBH) of all the trees bearing fruit; a vine-fruit index, calculated as the sum of DBHs of the host trees containing vines which were bearing fruit; and a total-fruit index, which was the sum of both these measures (Sommer *et al.,* 2011). For the purposes of this project a mean tree- vine- and total- fruit index was calculated using six years worth of data, for each month. Figure 2.2 shows the variation in the tree- and vine-fruit indices throughout the year.

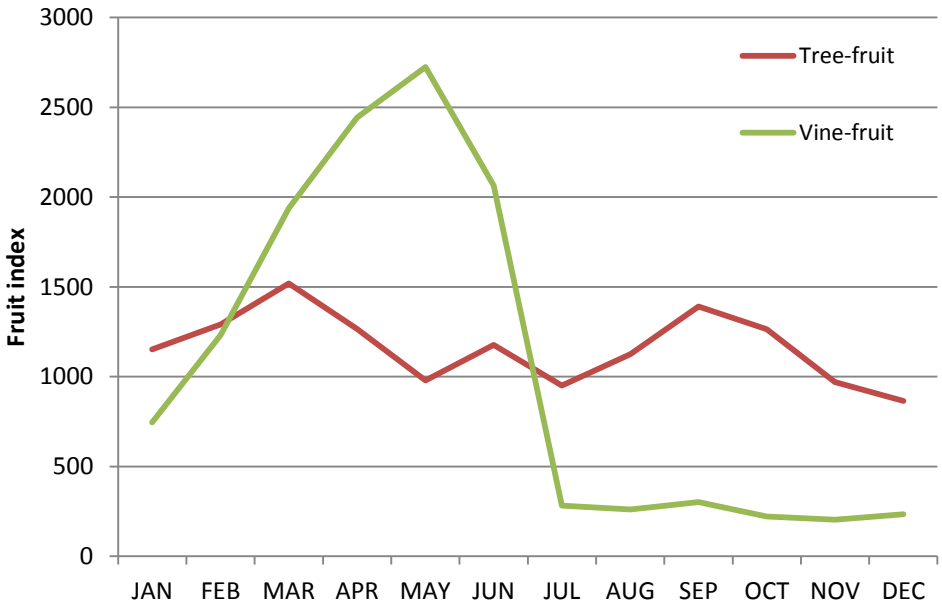

Figure 2.2 Average vine and tree fruit production throughout the year along transects at Kwano site.

The vine- and tree- fruit indices are un-correlated (Spearman's rank correlation r_s=0.406, p=0.191) and the pattern of production of the two fruit types throughout the year is quite different. Vine fruit production shows a rapid increase from mid way through the dry season (January) until it peaks at the end of the dry season/ beginning of the wet season (March-June). This peak is followed by a severe decline and then consistently low levels of vine fruit production during the rest of the wet season. In contrast the production of tree-fruit is more constant throughout the year with small peaks during March and September (figure 2.2). The overall contribution of the two types of fruit in the total-fruit index is similar (tree-fruit index: 52% total-fruit index) but total-fruit index is more strongly correlated with vine-fruit index (Spearman's rank correlation between total-fruit index and (a) tree-fruit index: r_s=0.600, p=0.088; and (b) vine-fruit index: r_s=0.883, p=0.002).

Intercorrelation between weather and fruit index variables

When investigating the influence of the various weather and fruit index variables on the other factors measured for this study it is important to consider whether there is intercorrelation between these variables, since this may have important implications for the interpretation of results. The relationships between the daily weather variables (including 30-day rainfall) and between the monthly weather and fruit index variables were assessed for both field sites (Kwano and Gamgam) using Spearman's rank correlations (tables 2.4 and 2.5).

As would be expected, significant positive correlations were found between certain temperature variables (tables 2.4. ad 2.5) and between certain rainfall variables (table 2.4). There was some evidence of a positive association between the rainfall and temperature variables (table 2.4), but more often the relationship between these two types of weather data was negative (tables 2.4. and 2.5). None of the rainfall measures correlated with the fruit index measures but several positive correlations between the fruit indices and the monthly temperatures measures were found (table 2.5).

Table 2.4. Relationships between daily weather variables. Results of Spearman's rank correlations are presented for Kwano site, in blue, above the diagonal line, and Gamgam site, in red, below the diagonal line.

Weather variable		Daily min temperature	Daily max temperature	Daily rain	30-day rain
Daily min temperature	r_s	**Kwano** / **Gamgam**	0.155	*0.107*	0.033
	p		**0.008**	***0.069***	0.571
Daily max temperature	r_s	0.156		-0.346	-0.536
	p	**0.007**		**<0.001**	**0.000**
Daily rain	r_s	0.014	-0.348		0.559
	p	0.808	**<0.001**		**<0.001**
30-day rain	r_s	-0.052	-0.470	0.551	
	p	0.375	**<0.001**	**<0.001**	

Table 2.5. Relationships between monthly weather and fruit index variables. Results of Spearman's rank correlations are presented for Kwano site, in blue, above the diagonal line, and Gamgam site, in red, below the diagonal line.

		Monthly mean min temperature	Monthly mean max temperature	Total monthly rainfall	Total-fruit index	Tree-fruit index	Vine-fruit index
Monthly mean min temperature	r_s	**Kwano** / **Gamgam**	0.370	-0.762	0.301	0.142	0.167
	p		0.327	**0.017**	0.431	0.715	0.667
Monthly mean max temperature	r_s	0.600		0.126	0.795	0.669	0.586
	p	***0.088***		0.748	**0.010**	**0.049**	***0.097***
Total monthly rainfall	r_s	-0.533	0.083		0.117	0.283	0.083
	p	0.139	0.831		0.765	0.460	0.831
Total-fruit index	r_s	0.600	1.000	0.083		0.600	0.883
	p	***0.088***	**<0.001**	0.831		***0.088***	**0.002**
Tree-fruit index	r_s	0.200	0.600	0.217	0.600		0.333
	p	0.606	***0.088***	0.576	***0.088***		0.381
Vine-fruit index	r_s	0.650	0.883	0.067	0.883	0.333	
	p	***0.058***	**0.002**	0.865	**0.002**	0.381	

2.2. Field work data collection methods

Two volunteer research assistants were recruited to assist with data collection for this project, one for each period of field work. Throughout the two field seasons alternate weeks were spent with each troop and each day a different adult female (n=16) acted as the focal animal. Focal animal observation was used rather than group observation because it is more effective as a method for collecting detailed feeding behaviour (National Research Council, 2003) and because the foraging group composition of the Gashaka baboons is known to vary throughout the day (Warren, 2003).

2.2.1. Behavioural Data collection

Each day, for 5 days a week, two focal animals were selected, usually from the same troop, and observed simultaneously with the support of two field assistants employed by GPP. The behavioural activities of the focal animals were recorded continuously throughout an eight hour observation day, which ran between 6:00 and 14:00 or 10:00 and 18:00 on alternate days. Early and late starts were used to ensure that the whole of

the baboon active period, from when they left their sleeping sites at around sunrise (c. 6:00) until they settled into a new sleeping site at around sunset (c.18:00), was represented by the data. Alternating early and late starts meant that following a late start, which ended at 18:00 at the baboons' sleeping site, the baboons could be found at the same location the following morning before they left their sleeping site. On late starts thorough searches were made of the troops' home ranges until the troop was found. The focal animal was chosen initially based on being the first adult female seen, but later in a week or month animals that had not recently been followed were selected for observation. If visual contact with the focal animal was lost for more than approximately two hours the focal observation was abandoned for the day.

Focal observations were recorded on a Psion Workabout handheld computer with Noldus Observer XT 8.0 software. This software enables the user to create study-specific configurations which allow detailed behavioural observations to be recorded in the field and later downloaded directly into the Observer PC software. For the purpose of this project a configuration was created for use with each of the two troops. Both of the configurations consisted of a behavioural channel, which contained 15 mutually exclusive activity states, most of which were also subdivided into behavioural modifiers. The structure of the configuration can been see in Table 2.6.

Table 2.6. Structure of Observer configuration for focal scans (based on Warren 2003). Continued over page.

Activity State	Behavioural Modifier	Definition
Rest	Inactive	Focal animal is inactive, eyes are open or closed
	Self groom	Resting animal picks though own fur
Cheek-pouch		Resting animal chews or manipulates food items from cheek pouches
Nursing		Resting animal allows dependent offspring to suckle
Travel	Walk	Walking movement of more than 1 ½ body lengths along the ground
	Run	Running movement of more than 1 ½ body lengths along the ground
	Climb	Movement of more than 1 ½ body lengths within vegetation

Table 2.6. Continued from previous page.

Activity State	Behavioural Modifier	Definition
Feeding	Various species	Selecting, processing and consuming any food item
	Various parts (e.g. seed)	
Give Grooming	ID of other animal	Picking through the fur of another individual with hands or mouth
Receive Grooming	ID of other animal	Focal animal is groomed by another individual
Aggression	ID of other animal	Any agonistic interactions, e.g. bite, chase, hit, given or received by focal animal
Play	ID of other animal	Engage in play with infant or juvenile
Copulation	ID of other animal	Mounting of female by male
Presenting	ID of other animal	Focal animal presents ano-genital area to another individual
Drink		Drinking
Observer directed		Barking, staring or performing other aggressive behaviours towards observers, which excludes all other behaviours
Other behaviour	Other social behaviour	Any other activity involving another troop member not defined here
	Other non-social behaviour	Any other activity not involving another animal, not defined here (e.g geophagy)
Out of Sight		Observer looses visual contact with the focal animal

The data collection regime meant that a maximum of 20 focal observations could be performed per troop per month. However, due to the difference in the number of focal animals between the two troops, this meant that the individual focal animals were more intensively sampled at Gamgam than at Kwano. In the second field work period (August-December 2009) the data collection regime was adjusted so that a maximum of 25 focal observations were performed on Kwano troop and 15 on Gamgam troop per month. The final result was that 53% of all the focal data collected was from Kwano and 47% from Gamgam; the mean number of focal hours spent with individual Kwano troop members was 51% of that spent with Gamgam troop members (see table 2.7 for absolute figures). At Kwano the number of focals per individual ranged between 7 and 11(Coefficient of variation [CV] = 14.81%) (not including KRM, 4 focals, who disappeared during the study period) and at Gamgam the number of focals ranged between 14 and 20 (CV=14.99%) (table 2.7).

Table 2.7. Summary of focal observation hours spent with each troop

			Mean (standard deviation)		
	Total focal time (hrs)	**Total no. focals**	**Focal length (hrs)**	**Focal hrs per individual**	**No. focals per individual**
Kwano	535.95	94	5.7(1.44)	48.72(12.03)	8.55(1.97)
Gamgam	473.72	84	5.64(1.39)	94.74(12.95)	16.80(2.59)
Both troops	1009.67	178	5.67(1.41)	63.10(25.03)	11.13(4.47)

The data for each focal day was uploaded into the Observer software as a single file. A function in Observer then calculated, for each file, the number of occurrences and the total duration of each behavioural category (activity state and behavioural modifier) which was then exported to Microsoft Office Excel 2007 for further analysis.

Food item specific feeding durations

Whenever feeding was observed, during the focal observations, the food item on which the focal animal was feeding was identified. Plant items were identified in terms of species and part (e.g. fruit or leaf) and non-plant items were identified in terms of more general categories e.g. 'fungus', 'ant'. Identification and application of scientific names from local names followed Warren (2003). For each focal observation day the amount of time the focal animal spent feeding on specific food items was recorded and used in the calculation of individuals' daily energy intake scores, detailed in section 2.4.2.

Food item specific feeding rates

Item specific feeding rates were obtained *ad libitum*, whenever it was possible to observe a feeding animal closely enough to count the number of items or the amount of a food item that was being eaten. Feeding rate observations were made only for adult females, the focal animals, but were not limited to the female that was acting as focal on any particular day. Details of how feeding rates were calculated for various food item

types are given in table 2.8. In total, 189 feeding rate observations were made for a total of 48 food items.

Table 2.8. Details of feeding rate determination protocols for different food types.

Food item or food item type	**Item counted during observation**	**Method for determining feeding rate**
Discrete items eaten whole (78% of feed rate observations)	Number of items (e.g. fruits, seeds, leaves)	Number of items eaten within a measured time period were counted.
Mangifera indica (Mango) fruit	Number of bites	Mass of fruit consumed with each bite estimated by observing bite sizes and then removing and weighing a piece of the fruit that appeared to be of an equivalent size.
Parkia biglobosa pericarp (spongy, yellow substance inside pod in which developing seeds are embedded)	Number of pods from which animal had eaten pericarp	Pods were collected and a mean mass of pericarp per pod (in g) was calculated.
Seeds from un-eaten pods where there were <50 seeds per pod (e.g. *Erythrophleum sauveolens*)	Number of pods from which animal had eaten seeds	Pods were collected and a mean value for the number of seeds per pod was calculated.
Seeds from un-eaten pods where there were >50 seeds per pod (e.g. Tuchi [local name])	Number of pods from which animal had eaten seeds	All calculations refer to number of pods rather than number of seeds eaten. When food item was collected for nutritional analysis only the seeds were collected but quantity is expressed as number of pods throughout all analyses. (Same for the kernels consumed from cobs of maize (*Zea mays*).
Small leaves	Baboon handfuls of leaves	Equivalent sized quantities of leaves collected and weighed and a mean mass per baboon handful was calculated.

The feeding rates, which consisted of the number of a particular item consumed per minute, were converted into mass feeding rates (i.e. the mass of food item consumed per minute) using the dry mass of the food items, measured as part of the nutritional analysis of sampled food items (see sections 2.3.3 and 2.4.2), and the following equation: mass feeding rate (g/min) = item feeding rate (no. items/min) x mass of item (g/item). Section 2.4.2 describes how appropriate feeding rates were assigned to the food items that the baboons consumed but for which feeding rate data were not available due, for example, to difficulties in making accurate enough feeding observations.

Social displacement behaviour

Data were also collected, on an *ad libitum* basis, on all aggressive, submissive and displacement interactions involving two focal animals (i.e. adult females only), including recording the 'winner' and 'loser' of each interaction. These data were subsequently pooled with equivalent data from other researchers who worked at Gashaka during the field period (N. Alberts, J. Rogge and G. Gordon) and from the records collected by the field assistants, giving a total of 211 and 342 interactions for Kwano and Gamgam troops respectively. Linear dominance hierarchies were then constructed by N. Alberts using the Landau's Linearity Index, corrected for unknown relationships. The resulting hierarchies were significantly linear for both troops (Kwano: h'=0.647, K=0.60, $p<0.001$; Gamgam: h'=1, K=1, p=0.008), although according to the criteria set out by Vervaecke *et al.* (2000) Kwano troop's hierarchy was not strongly linear (i.e. $h'<0.9$). The directional consistency index (DCI), which represents how unidirectional the dyadic relationships are, with a maximum value of 1 representing entirely unidirectional relationships (Vervaecke *et al.*, 2000), was also not very high, especially for Gamgam troop (Kwano DCI=0.83; Gamgam DCI=0.57). The focal animals were then grouped into high (Kwano n=4; Gamgam n=2), middle (Kwano n=4; Gamgam n=1) and low rank categories (Kwano n=3; Gamgam n=2), depending on their position in the hierarchy and these categories were used in all further analyses.

GPS data

A GPS (Global Positioning System, Garmin, GPSmap 60CSx) was carried during observations in order to obtain a measure of how far the focal animal travelled during the focal observation day. However, due to equipment limitations, this data was not available for every focal observation. The GPS was switched on as soon as the focal animal was located and identified, and switched off at the end of the focal day or when

visual contact with the focal animal was lost. The GPS calculated the distance travelled, moving time, stopped time, maximum speed, overall average speed and moving average speed of the observer during the observation day. The distance travelled was then used in the calculation of daily calculated energy expenditure (see section 2.4.2 below). Throughout most of each observation day the observer stayed within 10-20m of the focal animal which means that, although the distance travelled recorded by the GPS is that of the observer rather than the baboon, this measure provides an estimate of the baboons' travel distance. The values obtained will, however, tend to underestimate the baboons' actual day journey length since the observer would often follow a more direct path than that taken by a baboon, for example, when the baboon was foraging, and also because it does not account for the baboons' vertical movement when climbing trees. Despite this, the method of determining travel distance used here is likely to be more accurate than many alternative methods such as determining straight line distances between the animal's locations at regular intervals (Isbell *et al.*, 1999).

2.2.2 Sample Collection

Faecal sample collection

Faecal samples were collected on an *ad libitum* basis whenever an adult female was observed defaecating. Samples were collected from all adult females but only when the identity of the female was certain and when there was no contamination from urine or other faeces. The faecal bolus was first homogenised using a stick and a sub-sample of approximately 2g was taken and transferred into a leak proof collection bottle (Azlon 7BWH0030N) containing 10ml of >95% ethanol. Each sample was labelled with the identity of the female, the date and the time. Parafilm was added to the lids of each of the storage bottles to protect further against evaporation or leakage.

Urine sample collection

Urine samples were also collected on an *ad libitum* basis when an adult female was observed urinating, when the animal's ID was certain, when the sample was not contaminated with faeces or other urine and when collection was practical. Samples were collected using disposable plastic pipettes, usually from live vegetation on which the urine would collect into droplets and tend not to become contaminated with dirt. Occasionally samples were collected from dead leaves on the ground or from rocks and when this was the case this was noted. As much sample as possible was collected and immediately transferred to 1.5ml microcentrifuge tubes (Fisher Scientific), which were labelled with the date, collection time and ID of the animal and kept upright, embedded in foam until return to the field centre. Each evening 3 x 30µl of the sample was transferred, using a 10-100µl precision micro pipette (Fisherbrand) and disposable tips, to a specimen collection card (903 Protein Saver Cards, Whatman). The card was then labelled with the date, collection time and ID of the animal and stored in a plastic container containing several sachets of silica gel to ensure that the samples did not retain or pick up any moisture. The specimen collection cards consisted of a thick piece of filter paper with five circles and a wraparound card cover (Figure 2.3). Each circle held approximately 30µl of urine enabling a total of 150µl of urine to be held on each card. If more than 150µl of sediment free urine had been collected a second card was used to store the remaining urine. For any card which contained < 150µl the number of 30µl circles holding urine was noted so that the total amount of urine on each card was known.

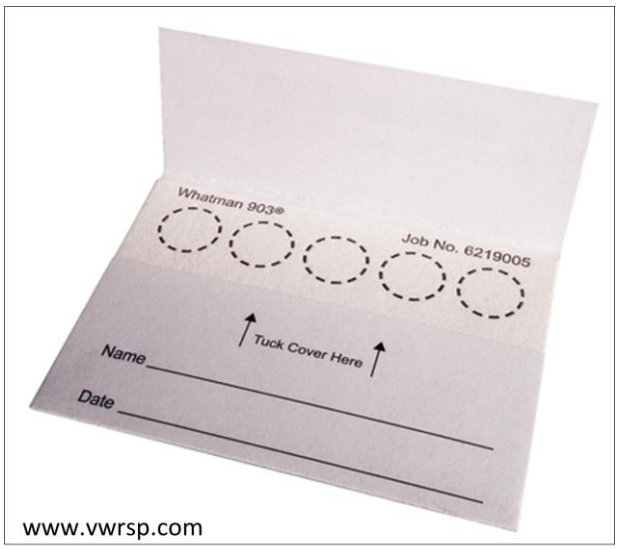

Figure 2.3. The 903 Whatman Protein Saver Card used for urine storage.

Faecal and urine samples were imported into the UK under DEFRA Products of Animal Origin licences (licence numbers: POAO/2009/044 and POAO/2009/438, appendix 2).

Plant food item sampling

Throughout the two fieldwork seasons and at both sites, samples of the baboons' most commonly eaten plant food items were collected. Where possible, if a food item was eaten at both sites, samples of that food item were taken from both sites. Efforts were made to select items as similar as possible to those selected by the baboons, for example in terms of ripeness and size. Where the baboons ate only part of a food item or where certain parts of the item were removed and discarded only the parts eaten by the baboons were collected. In general, food item samples were collected from several different plants of the species of interest, including, where possible, the actual plant that the baboons were observed feeding from. Sample collection took place as soon as possible after feeding had been observed. These procedures were followed because the phytochemical composition of plants is known to vary both in time and between individual specimens (Chapman *et al.*, 2003).

On the day of collection, after returning to the field station, food samples were prepared (e.g. fruit flesh removed from uneaten seeds, or nuts cracked and uneaten cases removed), cut into small pieces, to aid drying, and wet mass was obtained using a precision balance. Samples were then dried in aluminium foil bowls in a kerosene lamp-powered drying oven. The temperature of the oven was regularly monitored to ensure that it did not rise above 50^{o}C since temperatures >50^{o}C can irreversibly alter the chemical composition of plant items (Lucas *et al.*, 2003). Similar methods for drying primate food samples have been successfully used in many previous studies (e.g. Calvert, 1985; Barton *et al.,* 1993; Barton and Whiten, 1994; Conklin-Brittain *et al.,* 1998; Knott, 1998; Chapman and Chapman, 2002; Chapman *et al.,* 2003). In order to determine when the samples were completely dry, samples were re-weighed at intervals until subsequent weighings showed no change in mass. For most samples a wet mass of between 50g and 200g was collected in order to achieve the minimum 15g dry mass necessary for all the required nutritional analyses. In total, 52 different food item samples were collected with a mean wet mass of 101.2 ± 9.4g (mean ± standard error), resulting in a mean dry mass of 31.7 ± 3.1g.

Crop-raided food items

Due to difficulties gaining access to crop-fields and obtaining permission from farmers, I was unable to collect samples for the food items which the Gamgam baboons obtained via crop-raiding. However, I was able to obtain samples of the most important of these items, maize, mango and banana, in the UK (the Kwano troop also eat mangos from an abandoned farm within their range). I selected items that appeared to be as similar as possible, in terms of colour, size and ripeness, to those consumed by the baboons in Nigeria. I then prepared and dried the samples in a drying oven that maintained a constant temperature of 40^{o}C for three days, by which time subsequent weighings

indicated no change in mass. Although there may well be differences in the phytochemical composition between the maize, mangos and bananas grown in Nigeria and those available to purchase in the UK, this method did have the advantage over other methods (such as obtaining values from Food Tables) because the UK sampled items were processed and analysed in as similar a way as possible to those collected in Nigeria. Section 2.3.3 gives details of the nutritional analyses and how the energetic content of each sampled food item is calculated.

2.3. Laboratory Methods

2.3.1. Faecal sample analysis

Faecal samples were analysed for metabolites of progesterone and glucocorticoids using Enzyme-Immuno-Assays (EIAs).

Hormone extraction

Hormone metabolites were extracted from the faecal bolus into alcohol to enable EIA analysis. Extraction had begun as soon as the faecal samples were placed in ethanol at the time of collection but in order to ensure that the hormone metabolites were extracted from all parts of the sample a double extraction procedure was undertaken (Ziegler *et al.,* 2000). The efficiency of this method has been investigated using radioactive recovery by previous researchers working with faecal samples from the two study troops (Higham 2006). Higham (2006) found that recovery of the radioactive label was 87.0 % ± 8.1 after the first extraction step and 97.5% ±2.9 after the 2nd extraction step. This value of 97.5% was used as the Recovery Factor in the final calculation of the concentration of hormone metabolite per dry faecal mass for each sample (see equation 2.2) to control for the difference between the actual concentration present in the original sample and the amount successfully extracted into the supernatant and measured in the

assay. Double extraction protocols followed Higham (2006) and full details are given in appendix 3. After extraction, pre-weighed centrifuge tubes containing the solid faecal matter were dried at 40^{o}C and weighed periodically until their mass stabilised. At this point the original tube mass was subtracted from the current total mass to give the dry mass for each faecal sample.

Enzyme-Immuno-Assay principle and details of assays used

The basic principle of the EIA used in this study is a competition reaction between the hormone metabolite (the antigen) present in the faecal sample supernatant at an unknown concentration and a labelled antigen of known concentration (equation 2.1):

Equation 2.1. Competitive binding process

$$[Ab] + [Ag] + [Ag^*] \rightarrow [AbAg] + [AbAg^*] + [Ag] + [Ag^*]$$

Where Ab= antibody (known quantity), Ag = antigen (unknown quantity), Ag*labelled antigen (known quantity)

The labelled antigen competes with the un-labelled antigen in the sample solution to bind to an antibody, originally raised against the antigen, and its success in doing so is negatively related to the relative concentration of the un-labelled antigen. After any unbound antigen has been removed, by washing, streptavidin-horseradish peroxidise is added which binds with the bound labelled-antigen, followed by a chromogenic substrate (TMB) which binds to the bound streptavidin-horseradish peroxidise causing a change colour. The degree of colour change is therefore negatively related to the concentration of the un-labelled antigen in the sample. The precise concentration of the hormone in the sample can then be determined by measuring the optical density of the solution.

For the purpose of the current investigation these reactions took place on a plate consisting of 96 wells. In order to determine the exact relationship between the optical

density of the solution in one of the wells and the concentration of the hormone metabolite in that well it is necessary to run a series of the competitive reactions with solutions containing known concentrations of the antigen. A curve can then be created which describes the relationship between the optical density and the metabolite concentration. The precise concentration of the metabolite in each well can then be determined from its optical density using this curve.

In the current study, EIAs were used to determine the concentration of certain metabolites of progesterone and glucocorticoids in the faecal samples. A wide variety of progesterone metabolites is excreted in primate faeces; the one assayed in this study, 5B-Pregnane-3A-20A-Diol, or PdG, has been used and validated previously for faecal samples from this population (Higham *et al.*, 2008b). The glucocorticoid metabolite assayed in this study was 11b-dihydroxy-CM or 5β-Adiol, which has also been validated for use in baboons (A. Daspre, M. Heistermann, L. Rosetta and P.C. Lee pers. comm. 2007).

Assays were performed on microtitre plates (Fisher Scientific) coated with unspecific antibodies by technical staff at the University of Roehampton. Plates were stored at -20^{o}C and were allowed to come to room temperature before beginning the assay. Each 96-well plate enabled 33 samples to be analysed in duplicate with the remaining 30 cells containing Blanks, Zeros, Quality Controls and Standard Concentrations. The role and composition of each of these is described in Table 2.9 and the assay protocols are given in appendix 3.

Table 2.9. Properties of assay plate well components

Category	Role	Composition	No. wells
Blanks	Measure background absorption	100µl assay buffer[1], 50µl label	2
Zeros	Measure maximum absorption	50µl assay buffer, 50µl label, 50µl antibody	2
Quality Controls	Known high (QCH) and low (QCL) concentrations of the metabolite (PdG, pg/50µl: QCH=400, QCL=25; 5β-Adiol, pg/50µl: QCH=200, QCL=50). Subsamples of the same batch used on every assay plate as a measure of inter-assay reliability.	50µl QCH or QCL, 50µl label, 50µl steroid specific antibody	8
Standard Concentrations	Nine serial dilutions of stock standard metabolite solution diluted with assay buffer (PdG: 16000-6.25 pg/50µl; 5β-Adiol: 156-0.6 pg/50µl). Allows the creation of a standard curve of known concentrations from which the concentration of unknown samples can be determined.	50µl standard solution, 50µl label, 50µl steroid specific antibody	18
Samples	Doubly extracted supernatant of faecal samples of unknown metabolite concentration diluted with assay buffer to an appropriate concentration.	50µl sample supernatant, 50µl label, 50µl steroid specific antibody	66

1. Assay buffer consists of sodium carbonate anhydrous, sodium hydrogen carbonate, distilled water and 1% Bovine Serum Albumin (Albumin, Bovine, Fraction V Sigma A4503)

The analysis of samples was repeated if there was more than 10% variation between the duplicates and whole plates were repeated if there were more than five poor duplicates on the plate, if the standard curve was not the correct shape, if the blank did not have an optical density within 0.5-1.5, if the optical density of the zero was not between 0.6 and 1.5 or if the QCLs and QCHs were not within two standard deviations of the mean inter-assay variation.

Dilution and parallelism tests

Parallelism tests were carried out in order to determine whether the samples reacted to dilution similarly to the solution used to construct the standard curve i.e. whether they

diluted in parallel. For example, would a sample diluted by a factor of 4 be measured, using the curve, as 4 times weaker than the original? These tests also allowed determination of which dilution factors would be needed to ensure the hormone metabolite concentrations of the majority of samples fell within the linear range of the standard curve. Six samples were selected (detailed in table 2.10), which represented both troops and all three reproductive states (cycling, pregnant and lactating) and as such were expected to represent a broad range of progesterone and glucocorticoid concentrations.

Table 2.10. Details of faecal samples used in parallelism tests

Sample no.	**ID**	**Troop**	**Reproductive state**
5	DRK	Kwano	Cycling
67	MMK	Gamgam	Cycling
78	LDI	Kwano	Pregnant
125	LDI	Kwano	Lactating
175	KAN	Gamgam	Pregnant
190	KAN	Gamgam	Lactating

Each of these samples was diluted with assay buffer to give a series of five double dilutions which were then run on a plate alongside the usual standard curve. For both hormones, the samples diluted roughly in parallel with each other though not with the standard curve (figure 2.4).

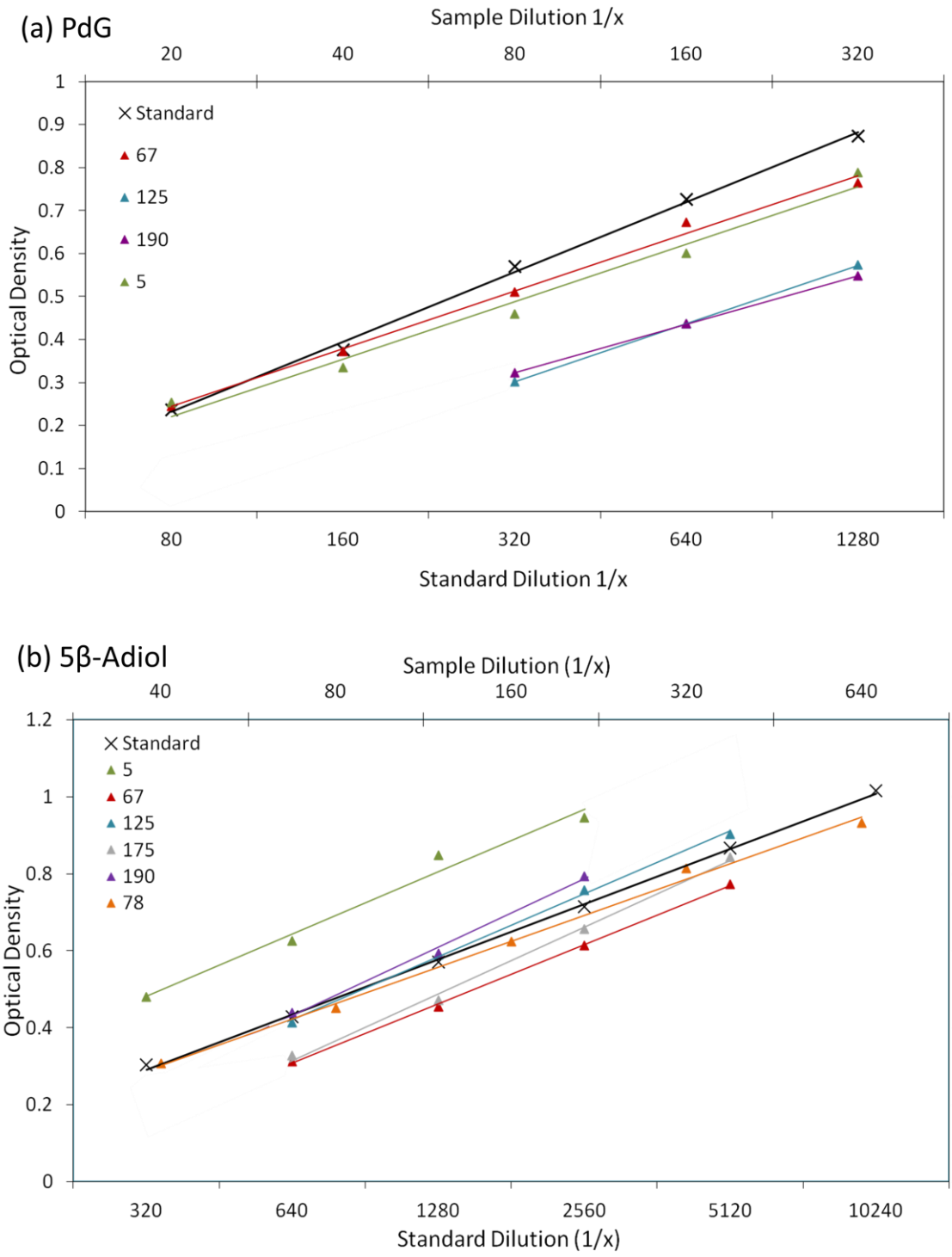

Figure 2.4. Parallelism charts for (a) progesterone metabolite (PdG) and (b) glucocorticoid metabolite (5βAdiol) assay. Triangles represent dilutions for samples 5, 67, 78, 125, 175 and 190 and crosses represent dilutions of the standard. For the PdG assay the results for two samples (78 and 175) are not included because only two dilutions resulted in values within the linear range of the standard curve.

Since the purpose of this investigation is to compare hormone levels between individual females and other broad categories rather than to detect small variations (e.g. in order to

detect ovulation) the small decrease in accuracy resulting from the lack of parallelism to the standard curve should not present a major problem especially since it is the relative differences in hormone levels that are of interest rather than the absolute values. The results of the parallelism tests also indicated that for both hormones the majority of samples could be run successfully at a concentration of 1/160. In practice, for PdG five different dilutions (1/20 - 1/320) were necessary to analyse all 230 faecal samples, although 93% of samples were analysed using one of two dilutions (1/80 and 1/160). For 5β-Adiol four dilutions were used (1/80 – 1/640) with 95% of samples analysed using one of two dilutions (1/160 and 1/320).

Intra- and inter- assay variation

Before beginning the analysis of samples, several plate tests were run to ensure that intra-assay variation was sufficiently low. For the plate test, metabolite solutions of known concentration were run in duplicate, 17 at a high (H) metabolite concentration and 16 at a low (L) metabolite concentration, in place of the samples. The co-efficients of variation for Hs and Ls were calculated using the formula: CV = (standard deviation / mean) x 100. Results of the final plate tests run using the PdG and 5β-Adiol assays are reported in table 2.11. Values of 10% and below are considered acceptable.

The high and low quality controls (QCH and QCL) run on each assay plate provide measures of inter-assay variation. The coefficient of variation (CV) was calculated using the two values of QCH and QCL from each plate. Results are reported in table 2.11. Values of around 15% and below are considered acceptable.

Table 2.11. Intra- and Inter-assay variation for PdG and 5β-Adiol assays

	Intra-assay Coefficient of variation		**Inter-assay Coefficient of variation**	
	H	**L**	**QCH**	**QCL**
PdG	5.63 % (n=17)	2.94 % (n=16)	8.56 % (n=28)	14.52 % (n=28)
5β-Adiol	3.40 % (n=17)	2.25 % (n=15)	10.36 % (n=23)	15.70 % (n=24)

Expressing hormone metabolite values relative to faecal weight

Equation 2.2 was used to convert the PdG and 5β-Adiol values obtained in the assays to a concentration relative to dry faecal weight which was the unit used in all further analyses.

Equation 2.2. Calculation of metabolite concentration in faecal samples

$$\text{Metabolite (ng/g dry faeces)} = \frac{\text{Dilution factor} \times \text{Extract volume (ml)} \times 1000 \times \text{Assay value (pg)}}{\text{Dry faecal weight (g)} \times \text{Sample volume (50ul)} \times \text{Recovery factor (97.5\%)} \times 1000}$$

Assay sensitivity

Assay sensitivity was determined by calculating the metabolite concentration at 90% binding. For the PdG assay this value was 7.94 pg/50µl and for the 5β Adiol this value was 0.42 pg/50µl. The linear range of the standard curve usually fell between 25 and 400 pg/50µl for the PdG assay and between 1.22 and 39pg/50µl for the 5β Adiol assay.

Control experiments

The efficiency of homogenisation, and sub-sample selection from the faecal bolus during collection was tested by comparing the results of PdG and 5β-Adiol assays performed on a number of samples where two sub-samples were taken from the same bolus (n=9). There was a highly significant correlation between the results of 5β-Adiol (Pearson correlation, $r=0.890$, d.f.=7, $p=0.001$) assays and PdG assays (Pearson Correlation, $r=0.987$, d.f.=7, $p<0.001$) for the pairs of sub-samples, as shown in figures 2.5 a and b.

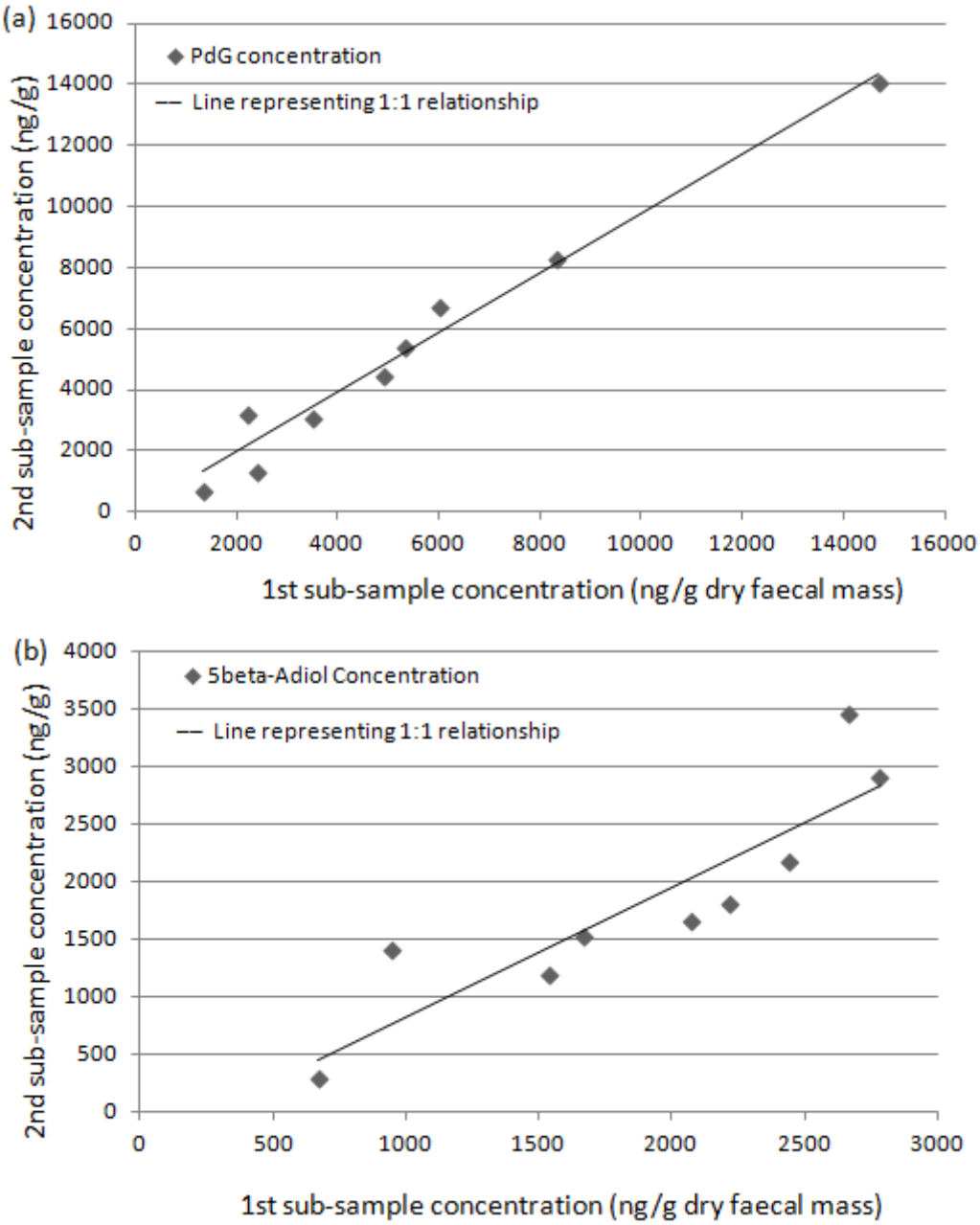

Figure 2.5. Comparison of the metabolite concentration in sub-samples of the same faecal bolus for (a) PdG and (b) 5β-Adiol.

2.3.2. Analysis of urine samples for urinary C-peptide (UCP) content

The concentration of urinary C-peptides (UCPs) in the dried urine samples was determined using IBL C-peptide ELISA (Enzyme-Linked Immuno-Sorbent Assay) kits (RE53011) designed for use on human serum, plasma and urine. The assay is able to detect C-peptides at concentrations between 0.06 and 16 ng/ml with a minimum detectable dose (MDD) of 0.064ng/ml (IBL International, 2009). Like the PdG and 5β-

Adiol EIAs used for the analysis of faecal samples this test is based on the principle of competitive binding. The contents of the kit and the purpose of each of its components are described in table 2.12.

Table 2.12. Description of IBL C-peptide ELISA enzyme immunoassay kit contents (IBL International, 2009)

Component	**Description**	**Role in assay**
Microtitre plates	Plates of 96 wells (12x 8 well strips) coated with non-specific anti-mouse antibody.	Competitive binding reaction takes place within each well. Specific antibody (Antiserum) binds to the non-specific antibody.
Antiserum	Monoclonal mouse anti C-peptide antibody ready to use.	The specific antibody to which the labelled antigen (Enzyme conjugate) and unlabelled antigen (C-peptide in urine sample) attempt to bind.
Enzyme Conjugate	Biotinylated C-peptide ready to use.	Labelled antigen which will compete with the un-labelled sample antigen to bind with the antibody (Antiserum).
Enzyme Complex	Contains horseradish peroxidase, ready to use.	Horseradish peroxidise binds with biotin on the bound, labelled antigen.
TMB substrate	Ready to use chromogenic substrate.	Turns blue on reaction with peroxidise (Enzyme Complex) bound to the biotin labelled antigen (Enzyme Conjugate).
TMB Stop	0.5M Sulphuric acid ready to use.	Stops the colour change reaction between TMB substrate and Enzyme Complex.
Standards	6 vials of lypholised C-peptide standards (0-16ng/ml) to be reconstituted with 0.75ml of distilled water.	Used to create the standard curve against which the c-peptide concentration of the urine samples can be determined.
Sample diluent	Solution for dilution of samples.	Used to reconstitute dried urine samples.
Wash Buffer (concentrated)	30ml solution diluted with 1170ml distilled water to make washing solution.	Used at two stages, 1^{st} to remove unbound antigen and 2^{nd} to remove unbound peroxidise (Enzyme Complex).

Sample preparation

The dried urine samples were reconstituted by soaking overnight, at 5^{o}C, in sample diluent. The filter paper section of each specimen collection card was removed from the card envelope and the circles containing urine, and the area around them into which any

urine had spread, were carefully cut around. One circle (30µl of urine) was put back in the storage box to be used later for creatine analysis, and the four other circles were cut into small pieces (c.3mm^2 square) and placed in a 1.5ml microcentrifuge tube. As the concentration of the original urine samples were unknown, the most concentrated solution, which provided sufficient volume was prepared by adding 720µl of sample diluent to the filter paper pieces, which resulted in a 1 in 6 dilution (120µl urine per 720µl diluent) of the original urine sample. The following day, the urine solution was transferred to another centrifuge tube and centrifuged in a Microcentrifuge (Sanyo Micro Centaur) at 13000 rpm for 2 minutes to ensure that the solution used in the assay contained no solid matter from the filter paper.

UCP assay protocol

Following the manufacturer's instructions (IBL International, 2009), the Wash Solution was diluted and the Standards reconstituted with distilled water (table 2.12). 100µl of the Standards and samples (urine solution) were then dispensed in duplicate into the appropriate wells (figure 2.6) using a precision pipette. 50µl of Antiserum, followed by 100µl of Enzyme Conjugate was dispensed into each well using the multipipette. The plate was then covered with cling-film and gently agitated on a plate shaker, where it was left to incubate, at room temperature, for 60 minutes. After this, the plate was washed on the plate-washer using the diluted Wash Solution and struck dry. 100µl of the Enzyme complex was then added to each well and the plate was left to incubate at room temperature for a further 30 minutes, after which is was again washed. Finally 100µl of TMB solution was added to each well, the plate was covered in cling-film and left to incubate in the dark, at room temperature for 20 minutes. The reaction was then stopped with the addition of 100µl of the Stop Solution. An automated plate reader (Multiskan Ascent, Thermo Labsystems) was then used to measure the optical density

of each well. The UCP concentration in each well was then determined from the standard curve by the Ascent software.

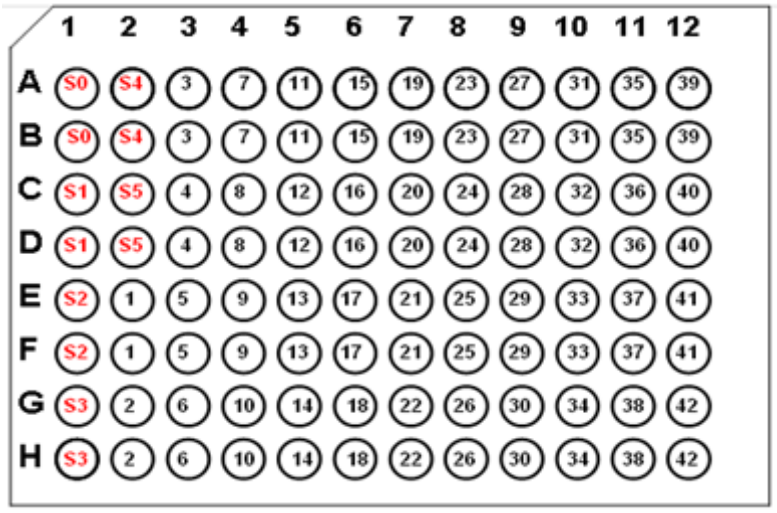

Figure 2.6. Layout of UCP assay plate. S0-7 = C-peptide Standards [0, 0.2, 0.7, 2, 6 and 16 ng/ml], 1-42= Urine samples

Creatinine analysis

Creatinine is a protein produced in muscle maintenance and excreted in urine at a constant rate. It can therefore be used as an indicator of urine concentration (Narayanan and Appleton, 1980). In the current study, the creatinine content of each urine sample was measured and UCP content was expressed relative to this value in order to control for variation in water content between samples.

Creatinine content was determined using an assay kit (Parameter™ Creatinine Assay, R&D Systems) which used the principle of the Jaffe reaction (Taussky, 1954), whereby the addition of alkaline picrate solution results in a colour change on contact with creatinine. The optical density of the solution is positively related to the creatinine content of the sample (R&D Systems, 2006). The kit consisted of 96 well microtitre plates (12 x 8 well strips), creatinine standard at 100mg/dl (to be diluted for a standard

curve), 0.13% picric acid solution and 1N sodium hydroxide. The minimum detectable dose for the assay is 0.02mg of creatinine per dl.

Creatinine assay protocol

The remaining filter paper circle from each specimen collection card was prepared in the same way as for the C-peptide assay and eluted overnight with 240µl of water, resulting in a 1 in 8 dilution (30µl urine per 240µl water). Following manufacturers' instructions (R&D Systems, 2006), the picric acid and sodium hydroxide were added together to form the Alkaline Picrate solution and the seven standards required for the standard curve were prepared via serial dilutions of the creatinine standard with distilled water. 50µl of standard, sample or distilled water was added to each well of the un-coated microtitre plate followed by 100µl of Alkaline Picrate solution (figure 2.7).

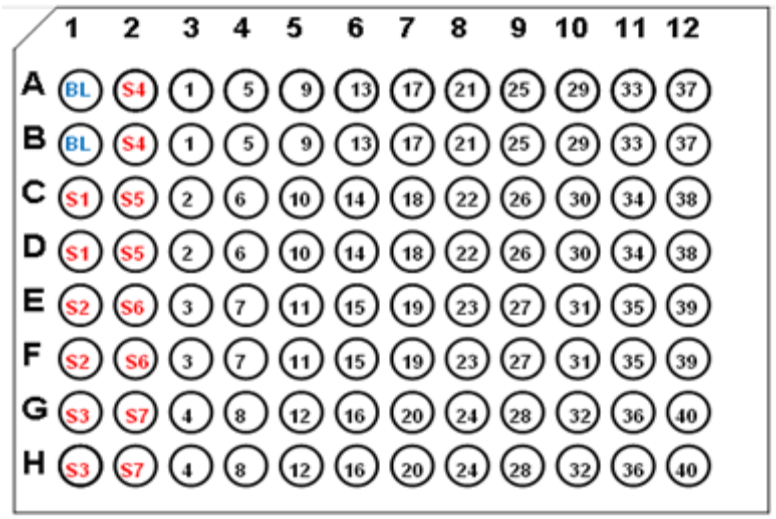

Figure 2.7. Layout of Creatinine assay plate. BL=Blank [distilled water], S1-7=Creatinine Standards [0.313, 0.62, 1.25, 2.5, 5, 10 and 20mg/dl], 1-40= Urine samples

The plate was then covered in cling-film and incubated on the plate mixer, at room temperature, for 30 minutes after which the optical density of each well was determined using the plate reader. The Ascent software was then used to create the standard curve and determine the creatinine concentration of each sample.

Expressing C-peptide concentration relative to creatinine content

The C-peptide value for each of the urine samples expressed as ng per ml of urine was converted to ng per mg of creatinine using equation 2.3:

Equation 2.3. Calculation of C-peptide in urine samples

$$\text{C-peptide (ng/mg creatinine)} = \frac{\text{C-peptide (ng/ml)}}{(\text{Creatinine [mg/dl]} / 100)}$$

As described in section 2.2.2 the urine collection protocol involved pipetting drops of urine from vegetation which usually meant there was only enough clean sample to apply to one protein saver card. For this reason, it was not possible to repeat the analysis of urine samples if the duplicates were not within 10% of each other or if the measurement did not fall within the linear range of the standard, as was possible for the hormone metabolites measured in faecal samples (section 2.3.1). Out of the 194 urine samples analysed for their UCP content 44 (22.7%) produced poor duplicates and 58 (30.0%) produced values that were below the sensitivity of the assay leaving 92 samples on which all further analyses are based.

Authors of previous studies have chosen to assign a C-peptide value equivalent to or just below the sensitivity of the assay to those samples in which UCPs could not be detected so as not to artificially eliminate samples with particularly low UCP values from their analyses (Deschner *et al.*, 2008; Emery Thompson and Knott, 2008; Girard-Buttoz *et al.*, 2011). However, for the current study it was decided to exclude samples in which UCPs were not detected from further analysis due to the fact that it was not possible to know whether the concentration of UCP in the samples was simply so low as to be beyond the detection limit of the assay or if there was a problem with detection of the UCP in these samples due either to contamination or incomplete elution from the

dried urine sample. The latter possibility is supported by the fact that some of the samples in which UCPs were not detected were taken from the same individual and on the same day as samples in which high UCP levels were detected.

2.3.3. Nutritional analysis of food samples

The nutritional analyses of the food item samples were performed at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin under the direction of Dr Sylvia Ortmann. In order to determine the physiologically available energy content of each sampled food item its macronutrient content was analysed using the following standard procedures. The Dumas combustion technique was used to determine nitrogen content and hence protein content, organic solvent extraction procedures were used to determine lipid content, Neutral Detergent Fraction procedures were used to determine fibre content and the non-structural carbohydrate content was determined via subtraction of the other components, plus ash, from the total dry mass of samples (Egan *et al.,* 1981; van Soest *et al.,* 1991; Knott, 1998; Conklin-Brittain *et al.,* 2006). These values were then combined to give the physiologically available energy content of each food item (see section 2.4.2 for calculations). Details of these procedures are given below and follow standard protocols for carrying out these analyses at IZW (S. Ortmann pers. comm.).

Sample preparation

Samples were ground using an electrical mill (IKA ® A11 Basic) in order to convert them into a powder fine enough to pass through a 1mm mesh, as required by the nutritional analysis procedures. Samples which proved difficult to grind due, for example, to a high lipid or sugar content, were frozen with liquid nitrogen and then ground.

Controls and duplicates

In order to ensure the accuracy and reliability of each of the nutritional analyses, control samples, for which the nutritional content was known, were run alongside the food item samples either on each analysis run or on each day. For most analyses a dried and ground sample of silver birch (*Betula pendula*) leaves was used but for lipid extraction a sample of dried animal feed (Kraftfutter) was used due to the high lipid content of the birch sample (S. Ortmann pers. comm.).

In each of the nutritional analyses the food item samples were analysed in duplicate, with the mean of these two values reported. Analyses were repeated if there was substantial deviation between the results of the two duplicates. The magnitude of deviation accepted was 10% unless otherwise stated in the sections below.

Determining dry matter

Following air drying in the field, samples were heat-dried at IZW in order to obtain a value for heat-dried matter mass. All the results of the nutritional analysis were reported relative to this value. Between 1 and 2 g of ground sample, in a pre-weighed ceramic cup, was dried at 100^{o}C for 24 hours. After this the cup was placed in a glass desiccator (Duran, Vakumfest) filled with a silica gel desiccant, allowed to come to room temperature and then weighed.

Determining inorganic matter (ash)

Following dry matter determination, the same portion of sample was used to determine the quantity of inorganic matter, or ash, in the sample. The sample was burnt at 540^{o}C for six hours until all the organic matter had been burnt off and only a residue of

inorganic matter remained. The sample was then cooled to room temperature in the desiccator and weighed.

Protein content

The protein content of each food item sample was inferred from its nitrogen content which was determined using the Dumas Combustion method (N'guessan *et al.,* 2009). The dried, ground samples were prepared for analysis by precisely weighing approximately 0.3g of each on tin foil. The sample and foil were then compressed into an air-free pellet. The nitrogen content of the sample was then determined using a fully automated Rapid N III Analyzer (Elementar Analysensysteme GmbH) which also estimated the protein content from the nitrogen content using a multiplication factor of 6.25 (N'guessan *et al.,* 2009). The Dumas Combustion method involves the combustion of the sample at high temperature (c. 950^{o}C) in the presence of catalysts which create oxides. Nitrogen oxides are then reduced to elemental nitrogen, aided by a copper catalyst, and separated from other gases including carbon dioxide which acts as the carrier gas. The Nitrogen content is then determined with a thermal conductivity detector. The efficacy of the analyser was tested after every ten samples by inserting a pellet of aspartic acid, for which the precise nitrogen content was known, followed by the control birch sample. Sample analysis was repeated if there was >5% deviation between the duplicates.

Lipids

The lipid content of the food item samples was determined via solvent extraction. The method involved a known mass of dried, ground sample being extracted with petroleum ether after which the solvent was distilled off and the residue of the sample was dried

and weighed. Lipid content was then calculated from the difference between the original sample mass and the final mass.

A fully automatic lipid extractor (Soxtherm Macro Extraction Unit, Gerhardt Laboratory Systems, Königswinter, Germany) was used which enabled three samples, in duplicate, to be measured simultaneously. Precisely 2g of dried, ground food item sample was weighed in a paper cup, dried at 100^{o}C for 1-2 hours and then cooled to room temperature in a desiccator. Metal holders were then placed into pre-dried and weighed extraction glasses containing 2-3 boiling stones. 140ml of petroleum ether was poured into each glass and the sample cups were placed into the metal holders. A ball of glass wool was placed in the top of each sample cup which ensured that the petroleum ether contacted the sample as a mist rather than as large drops. The extraction cups were then inserted into the extraction unit.

The extraction procedure consisted of a 30 minute boiling phase, a 40 minute extraction phase and a five minute distillation phase. On completion of the extraction procedure, the sample cups and holders were removed from the extraction glasses, which were then dried at 100^{o}C for one hour. The glasses were then placed in the desiccator for at least two hours after which they were weighed. The lipid content (W) of the sample could then be calculated using equation 2.4:

Equation 2.4. Calculation of lipid content

$$W = \frac{(m_2 - m_1) \times 100}{m_0}$$

Where: W=lipid content of sample; m_1 = mass of empty extraction glass with boiling stones; m_2 = mass of extraction glass with boiling stones and lipid after drying; m_0 = mass of original sample. All masses in grams.

Fibre

Fibre content was determined using the detergent system of fibre analysis (Van Soest *et al*., 1991), which involves the sequential dissolving of plant cell components in order to obtain values for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (figure 2.8).

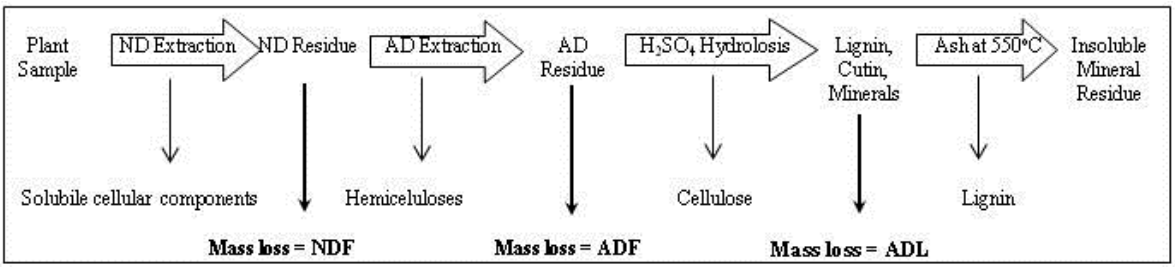

Figure 2.8. The detergent system of fibre analysis. Where: ND=Neutral Detergent, AD = Acid Detergent, F=Fibre, L=Lignin

Sample preparation for fibre analyses

Approximately 5g of each of the food item samples was placed into pre-weighed filter bags (Akom Technology F57 Filter bags) which were then sealed. Samples with a lipid content greater than 5 % (n=19) were soaked in acetone before the analysis in order to remove excess lipid, which can interfere with the NDF and ADF procedures (Van Soest *et al.* 1991). The NDF, ADF and ADL analyses were carried out for each sample but only the NDF procedure is detailed here since only this value was used to determine the physiologically available energy content of the samples. Details and the ADF and ADL procedures are given in appendix 4.

Neutral Detergent Fibre (NDF) procedure

The Neutral Detergent Solution was made by dissolving disodium EDTA dihydrate, Sodium teraborate decahydrate, Sodium lauryl Sulphate, 2-ethoxyethanol and Disodium hydrogen phosphate in distilled water. The digestion apparatus (Akom 200/220 Fiber

Analyzer) allowed 24 bags (11 samples in duplicate, one sample control and one empty filter bag control) to be analysed simultaneously, held in a bag suspender within the digestion vessel. The Neutral Detergent solution was added to the digestion vessel along with anhydrous sodium sulphate and heat-stable bacterial alpha-amylase and the samples were heated and agitated within the solution. After 75 minutes the solution was exhausted and the samples were rinsed within the digestion vessel, three times with boiling distilled water and alpha amylase, which was omitted for the final rinse. Each rinse lasted five minutes and the samples continued to be agitated throughout. The bag suspender was then removed from the vessel and the excess water was pressed out of the sample bags, which were then soaked in acetone for 3 minutes. After the acetone had completely evaporated the samples bags were dried at 100^{o}C for two hours, after which they were allowed to come to room temperature in a desiccator and then weighed. The ADF and ADL procedures were then carried out on the same sample bags after which they were burnt at 525^{o}C for 3 hours in pre-weighed ceramic beakers. The mass of the remaining ash was calculated after the ceramic beakers had returned to room temperature in the desiccator.

Calculating fibre content

Percentage of NDF of each sample were then calculated using equations 2.5a and b:

Equations 2.5. Calculation of NDF content

(a) Bag correction factor (C) $= \dfrac{Mb_{ag}\,(M_{burn} - M_{cup})}{Mb_{ag}}$

(b) NDF% $= \dfrac{(M_{NDF} - (Mb_{ag} \times C) - (M_{burn} - M_{cup})}{M_s} \times 100$

Mb_{ag} = Mass of sample bag, M_{burn} = Mass of sample and cup after burning, M_{cup} =Mass of sample cup, M_{NDF} = Mass of sample and bag after NDF analysis, M_s = Mass of sample

2.4. Calculations and statistical analysis

2.4.1. Activity budgets

Individual specific daily activity budgets were constructed from the continuous focal follow data (2.2.1) by pooling the original 15 mutually exclusive in-sight activity states into 5 general activity categories: travelling, resting, feeding, social behaviour, other behaviour (table 2.13). The number of hours that the focal animal spent in each of these 5 categories on a given focal observation day, the hourly activity budget, was used to determine calculated energy expenditure (section 2.4.2) and the percentage of total in-sight time spent in each of the 5 categories was also calculated to create percentage activity budgets for each focal day.

Table 2.13. Pooling of original activity states categories into five activity categories

Original activity states	**Activity budget category**
Rest	Rest
Cheek-pouch	
Nursing	
Travel	Travel
Feeding	Feeding
Give grooming	Social
Receive grooming	
Aggression	
Play	
Copulating	
Presenting	
Other social behaviour	
Drink	Other behaviour
Observer directed behaviour	
Other non-social behaviour	
Out of sight	Out of sight

2.4.2. Calculated energetic status measures

The hourly activity budgets, feeding rate observations and results from the nutritional analysis of food item samples were combined to give three calculated energetic status measures for each focal observation day: calculated energy balance, calculated energy

intake rate and calculated energy expenditure rate. The calculated energy balance of a focal animal on a given observation day was calculated by subtracting that animal's total calculated energy expenditure for that day from her total calculated energy intake. In addition, calculated energy intake and expenditure were standardised across focal days of different lengths by dividing the values by the total number of hours the focal animal was observed for during that day (the 'in-sight' hours) to give an intake and expenditure rate for each focal day. The methods for determining calculated energy expenditure and intake are given in the following sections.

Calculated Energy Expenditure

The calculated energy expenditure of the focal animal for each focal observation day was calculated by combining the hourly activity budget data (section 2.4.1) with activity specific energy constants, which have been used in previous publications in order to calculated energy expenditure by non-human primates (Leonard and Robertson, 1997; Key and Ross, 1999; N'guessan *et al.*, 2009). The energetic cost, in kcals, of each of the activity states, except travelling, was calculated using equation 2.6 (adjusted from Leonard and Robertson, 1997):

Equation 2.6. Calculation of energetic cost of activities

$A_i = D_i \times T_i \times (BMR/24)$

Where: A_i= Energetic cost of activity i during observation day (kcal); T_i= Hours of observation day spent performing activity i and D_i= the activity specific energy constant, where D_{Rest}=1.25, D_{feed}=1.38, D_{social}=2.35 and D_{other}=1.66 (the mean value of D_{Rest}, D_{feed} and D_{social}).

The same value of BMR, or basal metabolic rate (per day), was used for all focal animals and was calculated using a value for body mass (M) of 13.3kg (mean body mass from 116 free-ranging female *P. anubis* from three sources: Smith and Jungers, 1997) using Kleiber's (1961) equation:

Equation 2.7. Calculation of BMR

BMR (kcal/24hrs) = 70 x Body mass (kg)$^{0.75}$

∴ Female *P. anubis* BMR= 70 x 13.3$^{0.75}$ = 487.514 kcal/24hrs

The activity specific energy constants used here (equation 2.6) were derived from data from studies of humans in which indirect calorimetry was employed to determine the metabolic costs of a very large number of specific activities (Passmore and Durin, 1955). Coelho (1974) and Coelho *et al.*, (1976) selected certain values from this data set and adjusted them for body size in order to estimate the energy expended during a variety of activities by Old World (Sykes' monkey, baboon (sub-species not given)) and New World (Guatemalan black howler monkey, Geoffroy's spider monkey) monkeys. Leonard and Robertson (1997) adjusted these values further to give activity specific energy expenditure constants for five major primate activities including resting, feeding and socialising/playing, which are the values used in the current study.

The method of energy expenditure determination used in the current study may not, therefore, accurately represent the actual energy expenditure of the study animals due to the fact that the energy constants were derived from data from humans, whose active behaviours differ in many ways from baboons (e.g. differences in locomotion, social behaviours and digestion), and due to the lumping together of a variety of behaviours into three broad activity types: resting, feeding and social behaviour. The category social behaviour is a particularly broad one, encompassing energetically cheap but common behaviours (e.g. being groomed) as well as energetically costly but rare behaviours (e.g. aggression/ fighting).

Because of these sources of error, it is important to treat the energy expenditure estimations produced for this study with caution. However, given that the purpose of

this study is to make within study comparisons of energetic values rather than to determine accurate values of energy expenditure for comparison with other studies, these inaccuracies should not bias results as they should affect all individuals, throughout the year, in roughly the same way.

A more complex equation (equation 2.8) is required to calculate the energetic cost of travelling since the distance travelled during the observation day must also be taken into account (Taylor, 1970; Key and Ross, 1999).

Equation 2.8. Calculation of energetic cost of travel

$A_{Travel} = (0.041 \times M^{0.60} \times D + (0.029 \times M^{0.75}) \times T_{Travel}$

Where: D = Distance travelled during observation period in m, M=body mass kg (13.3kg) and T_{Travel} = Hours spent travelling.

An approximate measure of the distance travelled by the focal baboons during the focal follow was provided by data from the GPS carried by the observer (section 2.2.1). GPS data were not available for every focal observation day, due to equipment limitations, so for those days without (68/130 focals), average distance travelled values were assigned. There was a significant difference in mean distance travelled between months (ANOVA: Both troops: F=2.484, d.f.84, p=0.024) but not between individuals within troops (ANOVA: Kwano, F=0.893, d.f.40, p=0.551; Gamgam, F=1.367, d.f.42, p=0.264). There was a significant difference between the distance travelled calculated from focal days with an early start (c. 6:00) and focal days with a late start (c. 10:00) for Gamgam troop (t-test: t=-2.76, d.f.41, p=0.009) but not for Kwano troop (t-test: T=-0.138, d.f.40, p=0.891). For these reasons focal observation days without their own GPS data were assigned the mean value for the month/ troop at Kwano and the mean value for month/troop/focal start time at Gamgam.

The energetic cost of each activity type was then combined to give, for each observation day, a value for total daily calculated energy expenditure in kcal using equation 2.9 (Leonard and Robertson 1997, Key and Ross 1999). This value was then converted into kJ using the conversion factor 1kcal=4.184kJ and used in the calculation of daily calculated energy balance. The kJ value was also divided by the total number of in-sight hours for each focal observation day to give an energy expenditure rate value (kJ/hr) which enabled comparison between focal days of different lengths.

Equation 2.9. Calculation of daily energy expenditure

Daily calculated energy expenditure = $A_{travel} + A_{rest} + A_{feed} + A_{social} + A_{other}$

Where A_i = total energetic cost of activity i during observation day

Energetic intake

The daily energy intake of individual focal animals was calculated by combining the three types of food item specific data: feeding duration and rate (detailed in section 2.2.2) and physiologically available energetic content. The physiologically available energy content of each food item was calculated from its macronutrient content, determined from the nutritional analysis (section 2.3.3), using equation 2.10:

Equation 2.10. Calculation of physiologically available energy content

$$\text{Kilojoules per g of food} = \frac{(CP\times 14.23)+(TNC\times 16.40)+(Lipid\times 35.02)+(NDF\times 2.44)}{\text{Dry weight of sample (g)}}$$

Where: CP = crude protein, TNC = Total Non-structural Carbohydrates, NDF = Neutral Detergent Fibre

The factor by which each macronutrient is multiplied represents the amount of energy it provides per unit mass (kJ/g) and these values have been used in previous analyses of baboon diet (Altmann, 1998). The amount of energy available from fibre was calculated from the amount of energy it yields as a carbohydrate (16.40 kJ/g) minus that retained by the symbiotic bacteria that ferment it (4.184 kJ/g) (Conklin-Brittain *et al.,* 2006) and

multiplied by 0.2 since baboons are able to digest only 20% of the fibre in their diet (Stacey, 1986) which gives (16.40-4.184) x 0.20 = 2.44 kJ of energy per gram of fibre.

Assigning energy content and feeding rate values to un-sampled items

The baboons were observed to eat a total of 110 food items, 95 of which consisted of identified plant items for which the species was known either by its Latin (n=69) or local (n=12) name or of items that could be readily recognised but for which a name was not known (n=14). For these items a unique name was attached for the purposes of this study. For example, two distinctive and abundant small fruits were given the names 'red berry' and 'white berry' and were easily and consistently identified by all data collectors. These identified plant food items accounted for 83.4% and 72.8% of total feeding time for Kwano and Gamgam troops, respectively. Of the remaining 15 food items, 9 consisted of a plant item for which the species could not be identified reliably but the part was known (e.g. a flower of unknown species), five consisted of non-plant food items and one category was reserved for entirely unidentified items (table 2.14).

Table 2.14. Description of un-identified and non-plant food item categories

Category (n)	**Food item no.**	**Description**
Plant item (9)	110	Flower, species unknown
	111	Fruit, species unknown
	69	*Ficus* (fig) fruit, species unknown
	112	Leaf, species unknown
	113	Seed, species unknown
	114	Stem, species unknown
	115	Bark, species unknown
	116	Exudate, species unknown
	117	Underground storage organ/ root, species unknown
Non-plant item (5)	119	Fungus
	120	Ant
	121	Caterpillar
	122	Invertebrate picked from vegetation or ground
	123	Invertebrate picked from water or rocks in river
Entirely unidentified food item (1)	118	Unidentified item

Feeding on these 15 types of item accounted for 16.6% of Kwano troop's and 27.2% of Gamgam troop's total feeding time and so it was necessary to assign appropriate energetic content and feeding rate values to these items in order to estimate the calculated energy intake values for entire focal days. In addition, item specific energy content and feeding rate data were only available for around half of the 95 named plant food items (although those sampled accounted for 60-70% of total feeding time on named items), which meant that appropriate energetic content and feeding rate values also needed to be assigned to these named but un-sampled items. The main techniques used to assign these values are described in the following section and full details, along with all the measured and assigned values, are presented appendix 5, table A5.ii-iii.

Mean values

Most plant food items for which the plant part (e.g. fruit) was known were assigned mean energetic content and mass feeding rate values for the appropriate plant part. Mass feeding rates for fruits were divided into small (<2cm diameter) medium (2-5cm) and large (>5cm) sized fruits in order to account for the different feeding rates known to be associated with these different sized items (pers. obs.). Certain items for which a specific feeding rate was not obtained but which were known to be eaten at a relatively slow rate (e.g. crop-raided USOs and bark) were, for this reason, assigned the lower quartile value calculated from all observed feeding rates rather than the mean value (appendix 5, table A5.iii).

Values from the literature

Although 6.1% of Kwano troop's and 10.6% of Gamgam troop total feeding time was spent on non-plant food items, in the form of fungi and invertebrates, these items were not sampled due to problems associated with their identification, collection and

preservation. Item feeding rates (e.g. number of ants eaten per minute) were obtained but these could not be converted in to mass feeding rates (g/min) since samples were not available from which to determine the dry mass of individual items. Dry mass and energetic content data were therefore obtained from relevant literature detailed in appendix 5, table A5.i.

Un-sampled crop-raided items (i.e. cassava, sweet potato and cocoyam, see table A5.i) were also assigned values from the literature, specifically from Wu Leung's (1968) "Food Composition Tables for Use in Africa". This resource provided values for the physiologically available energy content of certain crop foods grown in Africa. Food items from two particularly difficult to sample plant food categories, exudate and bark, were also assigned values from the literature. The energy content and feeding rate values for exudates came from values for 'Fever tree (*Acacia xanthophloea*) gum' from Altmann's (1998) study of yearling yellow baboon feeding behaviour in Kenya. The bark energy content value came from Calvert's (1985) study of West African gorilla feeding behaviour and was calculated from the macronutrient content of bark from two tree species eaten by the gorillas.

Values from similar items

A few specific items, which could not be sampled, were assigned energy content or feeding rate values from very similar items. For example, a common fruit from the *Landolphia* genus known by the local name 'Small Tiboko' was assigned the average energy content value from two very similar fruits known as Medium and Large 'Tiboko'. A readily recognised but previously unnamed plant which was given the name 'small pod', for the seed cases that the baboons picked from it, was assigned the feeding

rate value from a plant known locally as 'Tuchi' which bore similar small pods (see appendix 5, table A5.i for all cases and details).

Calculations for determining daily energetic intake

The physiologically available energy content of each food item, and the values assigned to un-analysed food items, were then combined with the item specific feeding duration and rate data to give a value for calculated energy intake for each focal observation day using equation 2.11:

Equation 2.11. Calculating energy intake for a focal observation

$$\text{Calculated energy intake} = \sum_{i} (X_i Y_i Z_i)$$

Where summation is taken over all food items (i=1,2,3…) and X= kJ per gram of food item, Y= feeding rate for food item (g/min) and Z= minutes spent feeding on food item

This value was then used alongside the calculated energy expenditure value to determine a calculated energy balance value for each focal day. The calculated energy intake value was also divided by the total number of in-sight hours for each focal observation day to give an energy intake rate value which enabled comparison between focal days of different lengths.

2.4.3. Statistical analyses

Much of the data analysis for this project involved building Generalised Linear Mixed Models (GLMMs), fitted using iterative generalized least squares (IGLS), in the statistics programme *MLwiN* 2.0. These models were used to analyse data such as the activity budgets and calculated energetic status measures, where each data point corresponded to one focal day, and the hormone metabolite and UCP data, where one data point corresponded to one faecal or urine sample. Both these types of data involved

non-independent data points due to the fact that multiple points (i.e. focal days or samples) were taken from each of the 16 focal animals. In order to control for this non-independence, hierarchical GLMMs were built with focal or sample number entered as one random effect nested within each individual, whose ID was entered into the model as a second random effect. All other variables of interest, such as troop, reproductive state, rainfall or temperature, were entered into the models as fixed effects so that their effect on the dependent variable, e.g. calculated energy balance or UCP level, could be assessed whilst controlling for differences between individual animals. When necessary, the dependent variable was Log_{10} transformed in order to achieve the normal and homoscedastic distribution of residuals required by this statistical method (Rasbash *et al.*, 2004).

The variation in the dependent variable explained by the model, or the model's fit, was assessed by performing the likelihood ratio test. The likelihood ratio test compares the fit of a model relative to a simpler model, often a null model, to assess whether extra variables in the more complex model have significantly improved its fit. A null model is a model that contains no explanatory variables (i.e. fixed factors) but which does contain random factors. Two types of null model are discussed in this thesis: a 1-factor null model, which contains only the first random factor (sample/focal number), and therefore does not control for the non-independence of samples from the same individual, and a 2-factor null model, which contains both the first and second (ID) random factors. The fit of the 2-factor null models is compared to the fit of the 1-factor null model using the likelihood ratio test in order to determine whether a significant amount of the variation in the dependent variables dataset is due to differences between individuals. The 2-factor null model is then built up by adding fixed factors and after each addition the model is compared, using the likelihood ratio test, to the 2-level null

model or to a model containing just one fewer fixed factor, in order to determine whether the addition of the newest fixed factor significantly improves the fit of the model.

The likelihood ratio test involves calculation of the test statistic, D, using equation 2.12, with a larger D value indicating a larger improvement in the model's fit. D is then compared to a χ^2 distribution to determine whether the improvement in fit is significant given the number of extra parameters in the model.

Equation 2.12. Calculation of likelihood ratio test statistic

D= (-2 x LogLikelihood of more complex model) - (-2 x LogLikelihood of simpler model).

Another test statistic, z, was calculated in order to determine whether the average relationship between any one of the fixed effects and the dependent variable was significant, e.g. the relationship between calculated energy balance and rainfall. Equation 2.14 was used to calculate z, which was then compared to a standard normal distribution to determine whether or not it differed significantly from zero, which, if it did, would mean that the relationship being examined was significant.

Equation 2.13. Calculation of z test statistic

$$z = \frac{\text{Coefficient}}{\text{Standard Error of Coefficient}}$$

Where: C=coefficient associated with the fixed variable of interest. For continuous variables this value represents the slope of the relationship. S.E.=standard error of the coefficient.

An example of one of these GLMMs is given in figure 2.9 to illustrate the use of these statistics. The dependent variable in both models 'GCLog10' is faecal glucocorticoid concentration Log_{10} transformed, henceforth referred to as 'GC level'. The two random factors are faecal sample number, represented by '*i*' and ID represented by '*j*'. In this

model there is just one fixed factor, Rain30day, which is the total rainfall that fell in the 30 days prior to the collection of the faecal sample from which the GC content was measured, henceforth referred to as 'rainfall'.

$$GCLog10_{ij} = \beta_{0j} + 0.000390(0.000099)Rain30day_{ij} + e_{ij}$$
$$\beta_{0j} = 3.053727(0.047957) + u_{0j}$$

$$\sigma^2_{u0} = 0.019080(0.008561)$$
$$\sigma^2_e = 0.059229(0.005997)$$
$$-2*loglikelihood = 27.940219(211 \text{ of } 211 \text{ cases in use})$$

Figure 2.9. Equation representing glucocorticoid GLMM containing 30-day rainfall (copied from MLwIN output). Where: $GCLog10_{ij}$ = the value of the dependent variable, GC level, for faecal sample *i* from individual *j*; β_{0j} = a coefficient representing the mean GC level across all individuals; $Rain30day_{ij}$ = the value of the fixed factor, rainfall, for sample *i* from individual *j* (the green number is the coefficient of the relationship between GC level and rainfall across all individuals); u_{0j} = error term representing the deviation of indivudal *j*'s mean GC level from the overall mean (β_{0j}); e_{ij} = error term representing the deviation of sample *i* from individual *j*'s mean GC level; σ^2_{u0} = between individual variance in GC level; σ^2_e = between sample, within individual variance in GC level. Numbers in brackets are standard errors.

The coefficient of the relationship between GC level and rainfall (0.000390) indicates that this relationship is positive and whether or not it is significant can be determined by calculating the z statistic: z= Coefficient/ SE ∴ z = 0.000390 / 0.000099 = 3.94, which when compared to a standard normal distribution corresponds to $p<0.001$ and hence is significant.

Figure 2.10 represents a similar model to the one presented in figure 2.9 but to which another fixed variable, in this case a categorical variable representing troop, has been added. The interaction effect between rainfall and troop is also included (Gamgam.Rain30day).

$$GCLog10_{ij} = \beta_{0j} + 0.000414(0.000147)Rain30day_{ij} + -0.209921(0.072923)Gamgam_j + -0.000082(0.000199)Gamgam.Rain30day_{ij} + e_{ij}$$

$$\beta_{0j} = 3.126215(0.054492) + u_{0j}$$

$$\sigma^2_{u0} = 0.003860(0.003070)$$

$$\sigma^2_e = 0.060158(0.006080)$$

$$-2*loglikelihood = 15.190789(211 \text{ of } 211 \text{ cases in use})$$

Figure 2.10. Equation representing glucocorticoid GLMM containing 30-day rainfall, troop and their interaction (copied from MLwIN output)

When categorical variables are included in these models, one of the categories, in this case Kwano troop, acts as the reference category to which values associated with the other category, in this case Gamgam troop, are compared. The number presented in this model as the Gamgam coefficient represents the fact that the mean GC Log_{10} value of Gamgam troop members is -0.210 lower than the average value for Kwano troop members, when rainfall is held constant, which, according to the z statistic (z = -0.210 / 0.0729 = 2.88 which corresponds to p=0.004) is a significant difference. The number presented as the rain coefficient is the slope for Kwano troop only, which is also significant (0.000414/0.000147=2.816, p=0.005). The model can be adjusted so that Gamgam troop acts as the reference category so that the relationship between GC level and rainfall can be determined for this troop. This example demonstrates the usefulness of the z test. If a model contains only one fixed factor then the z test provides little more information than a likelihood ratio test comparing it to the null model, since both tests describe whether or not the fixed factor is significantly related, or significantly affects, the dependent variable. However, if the model contains more than one fixed factor the z test is useful for examining whether each of the variables is significant when the other is held constant or, if categorical variables are involved, for examining the relationship for the different categories within the variable.

We can determine whether this second, more complex, model (figure 2.10) fits the GC dataset significantly better than the first model (figure 2.9) by calculating the likelihood test statistic: D = -2*logliklihood of more simple model - -2*logliklihood of more complex model = 27.940 - 15.191 = 12.749. When compared with a χ^2 distribution with 2 degrees of freedom (because two new parameters, green numbers, have been added to the model) this value of D corresponds to p = 0.0017. We can therefore conclude that the addition of troop, and its interaction with rainfall, to the model significantly improves its fit.

Throughout this thesis these relationships are frequently presented in the form of a scatter graph where the actual sample values are presented as points and the linear relationship between the fixed and dependent variable, as predicted by the model, is presented as a best fit line. Solid lines indicate a significant relationship ($p<0.05$) and dashed lines indicate a marginally non-significant trend ($p>0.05$ but <0.10). The inclusion of the random factor ID in the models means that a different linear relationship between e.g. GC level and rainfall is predicted for each individual. The slope of the relationship will be the same for each individual (or if e.g. troop is included as a fixed factor in the model, for each individual within a troop) but the intercept will vary. Figure 2.11a shows the relationship between GC level and rainfall for each individual as predicted by the model given in figure 2.10 whereas figure 2.11b shows the average relationship for each troop from the same model. The second figure clearly demonstrates that there is a significant difference between the GC levels of the two troops, when rainfall is controlled for, a relationship which was not so clear from the first figure. When the results of GLMMs are presented graphically in this thesis, average linear relationships are presented rather than separate relationships for each individual.

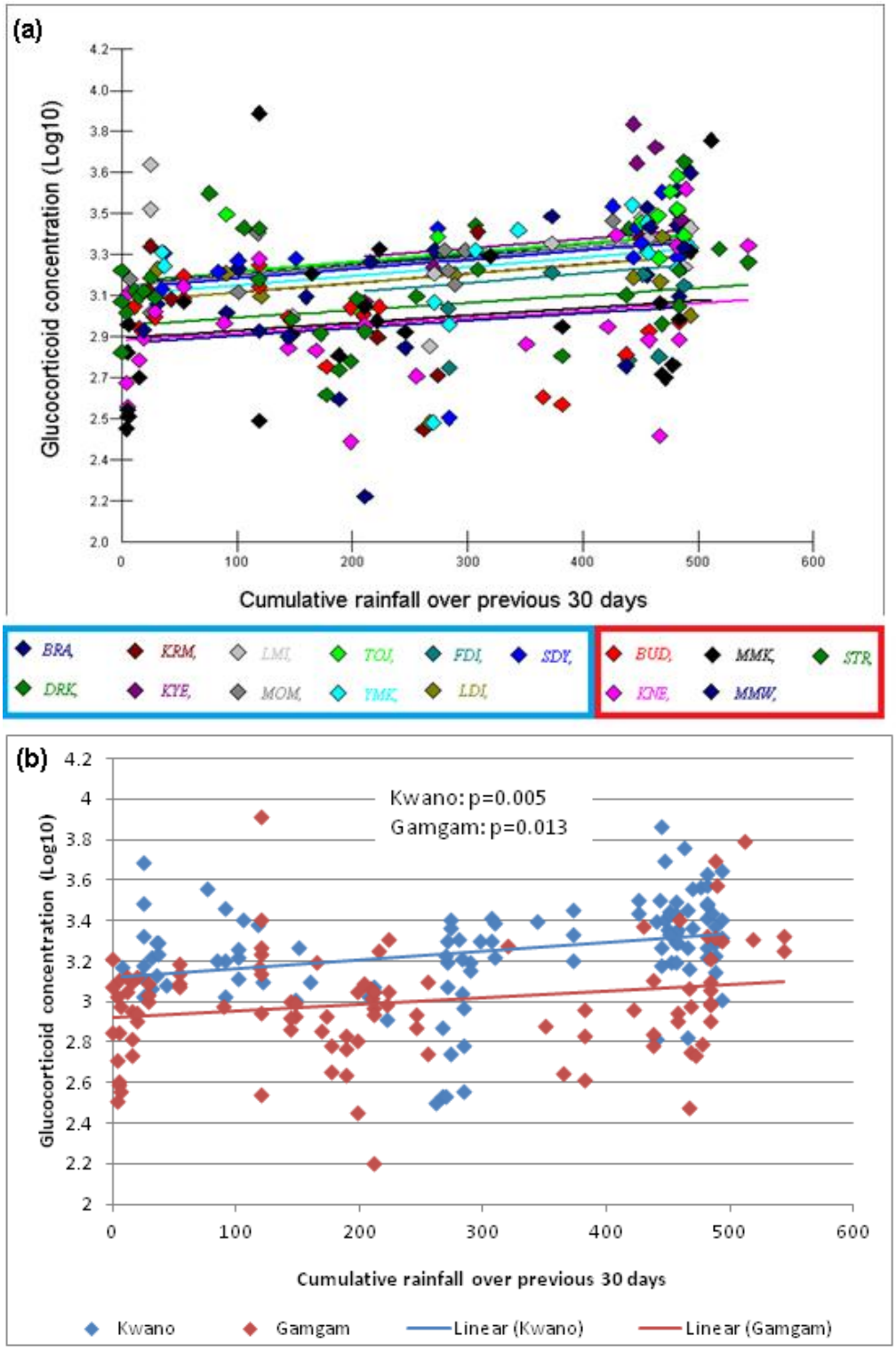

Figure 2.11. Graphical representation of glucocorticoid GLMM containing 30day rainfall, troop and their interaction effect. Points represent individual data points, lines represent lines of best fit. Glucocorticoid concentration in ng/g dry faeces, rainfall in mm. (a) Shows linear relationships for all individuals (three letter IDs given) and (b) shows average linear relationships for the two troops.

Data are also frequently presented in the form of box plots. In these plots the central line of each box represents the median value of the dataset, the boxed region represents the interquartile range of the dataset and the whiskers represent the upper and lower quartiles. Outlying data points are presented as circles or asterisks (Figure 2.12).

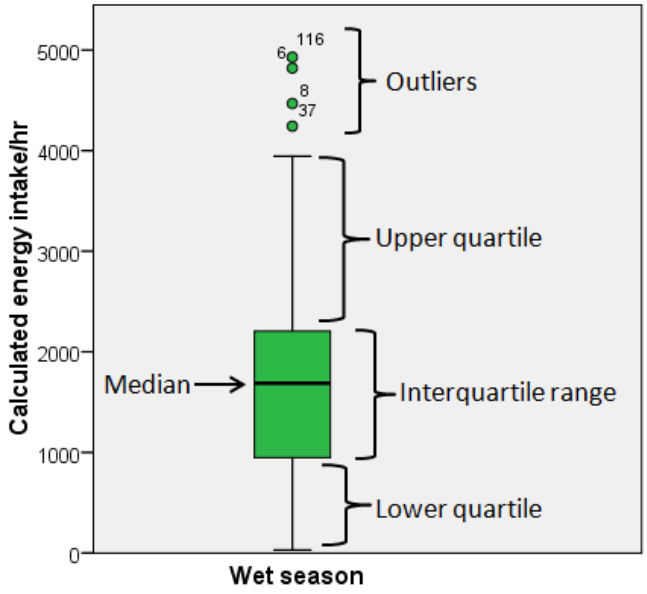

Figure 2.12. Diagram showing properties of box-plots presented in this thesis

At various points throughout this thesis the results of other standard parametric and non-parametric statistics are presented including Pearson's product moment and Spearman's rank correlations, Mann-Whitney U tests, Kruskal Wallis tests and ANOVAs, all of which were performed in SPSS (version PSAW 17). Non-normally distributed data were Log_{10} transformed before using parametric tests. The level of significance was taken at $p<0.05$ with $p<0.01$ considered highly significant and $p>0.05$ but <0.1 usually treated as marginally non-significant. Two-tailed tests were used throughout.

Chapter 3

ACTIVITY BUDGETS AND CALCULATED ENERGY BALANCE

3.1. Introduction

3.1.1. Activity budgets

Activity budgets describe how an animal partitions its time between various types of activities, most commonly: feeding, travelling, social behaviours and resting. The factors which influence primate activity budgets have been well documented, especially in baboons (e.g. Dunbar, 1992). An animal's activity budget is primarily influenced by the climate, habitat and the length of its active day. For diurnal species this will be the hours of daylight (Altmann and Muruthi, 1988; Dunbar, 1992). More specifically, feeding or foraging time is influenced by individual energetic and nutritional requirements (influenced by body size, reproductive state, age, thermoregulation costs, habitat and food item quality etc. [section 1.2]); travelling time is influenced by resource availability and distribution; social time is influenced by group size, food distribution and the value of maintaining alliances; and rest time generally consists of the time that remains after allocation to all other essential activities, although a limited amount of rest time is necessary for physiological processes such as digestion (Altmann, 1974; Altmann and Muruthi, 1988; Dunbar, 1988; Dunbar, 1992). A few specific factors, relevant to the current study, which are known to affect primate activity budgets are discussed in the following section.

3.1.1.1. Food-enhancement

Food-enhancement affects activity budgets by increasing foraging efficiency, since high quality, clumped and abundant food sources are available. The result of this is generally a decrease in the amount of time devoted to feeding and foraging, which in turn leads to

changes in other aspects of the activity budget due to the extra time available (Altmann and Muruthi, 1988; Forthman-Quick, 1986; Strum, 1994). Specifically, resting time tends to increase in food-enhanced primates, and increases in time devoted to social behaviours have also been recorded (e.g. Fa, 1991 but see Forthman-Quick, 1986). The effect of food-enhancement on travel time is less clear cut, although intuitively travel time would be expected to decrease if an abundant, high quality, clumped food resource becomes available, the availability of non-food resources such as water and sleeping sites, which are unaffected by food-enhancement, also influences travel time. Animals may also have to travel to reach food-enhancement sites, especially if they are associated with some kind of risk (as with crop-raiding) which means the animals are unable to spend most of their time near to them. Although many previous studies have demonstrated a decrease in travel time as a result of food-enhancement, others have not (e.g. Altmann and Muruthi, 1988; Eley *et al.*, 1989).

As discussed in section 1.2.2, the effect of crop-raiding on activity budgets is likely to be less than other, more well studied, forms of food-enhancement such as deliberate provisioning or rubbish-raiding. Crops may not be available all the year round and, like wild-foods, will be influenced by climatic factors such as rainfall so the benefits may be less than other forms of food-enhancement. In addition the risks and costs will be greater. Crop-raiding involves direct conflict with humans (i.e. farmers) which may result in, for example, increased travel time due to animals running away from the farmer as well as time spent travelling between the crops and safe refuges (Warren, 2003; Higham, 2006).

3.1.1.2. Effect of season

In tropical regions, such as those inhabited by baboons, large variation in rainfall throughout the year tends to result in distinct wet and dry seasons. The availability of plant foods to primates varies accordingly with higher productivity and greater food availability in the wet season contrasting with vegetation die off and decreased food quality in the dry season (Dunbar, 1992; van Soest, 1982; Toledo *et al.*, 2011). This variation is expected to affect primate activity budgets since, if food quality and abundance are lower, more time must be spent seeking for, processing and consuming foods and therefore less time is available for other activity budget components, namely resting and social behaviour (Dunbar, 1992). Food-enhancement can act to buffer environmental influences, such as the variation between seasons, by providing a food source which does not vary with weather patterns as do wild-foods (Bronikowski and Altmann, 1996). However, the extent of this buffering will vary depending on the type of food-enhancement, for example the availability of rubbish-raided foods is less likely to be influenced by environmental factors than that of crop-raided foods (Higham *et al.*, 2009a).

3.1.1.3. Effect of rank

A female primate's position in a social hierarchy, or her rank, may affect her activity budget due to the priority access to resources that dominant individuals enjoy over subordinates (Barton, 1993; Barton and Whiten, 1993; Altmann and Alberts, 2005). This may mean lower ranking animals need to spend more time feeding and travelling than high ranking animals, in order to obtain approximately the same amount of food (Isbell and Young, 1993).

3.1.1.4. Effect of reproductive state

Reproductive state is expected to influence activity budgets due to the increased energetic demands associated with pregnancy and lactation (section 1.1.2.). Pregnant and lactating females are known to increase their food consumption, relative to cycling females (Silk, 1987; National Research Council, 2003) or alternatively reduce activity levels in order to conserve energy (Altmann, 1980; Barrett *et al.*, 2006).

3.1.2. Estimating energy balance in wild animals

3.1.2.1. Importance of estimating energy balance

If an animal is to survive and reproduce it must ensure that it balances the energy it expends, in order to maintain homeostasis, find food, engage in social interactions and reproduce, with the energy it takes in from food (Lindström, 1999). If an individual fails to take in sufficient energy to balance these costs, it will be in negative energy balance, which in the long term can lead to energy stores being used up; to the re-direction of energy away from growth and reproductive functions to essential body maintenance; and eventually to death if the negative energy balance is particularly severe or sustained (e.g. Schneider and Wade, 2000). An individual's energy balance is therefore tightly linked to its physiology, growth, reproductive success, longevity and behaviour (Charnov and Berrigan, 1993; Sherry and Ellison, 2007). Inter-individual variation in energy balance will affect individual condition and reproductive success, a good example of this is the effect of rank on feeding behaviour and reproductive success in baboons. As discussed in sections 1.2.3 and 3.2.1 higher ranking females enjoy better access to high quality feeding sites (olive baboons: Barton, 1993) and foods (yellow baboons: Altmann and Alberts, 2005) than lower ranking females as well as higher rates of nutrient acquisition (Chacma baboons: Barton and Whiten, 1993). Alongside the

energetic benefits of high rank is increased reproductive success: high ranking females have shorter inter-birth intervals than low ranking females (Chacma baboon: Bulger and Hamilton, 1987) and produce offspring which grow faster and are larger as juveniles (Johnson, 2003). Temporal variation in energy balance can also be important since it can affect the timing of life history events such as reproduction (Koenig, *et al.*1997). For example, in a study of Bornean orangutans, Knott (1998; 2001) demonstrated an association between severe food shortages and both reduced energy intake and lowered oestrogen levels in non-pregnant females, factors associated with decreased probability of conception, alongside a decrease in the record of conceptions. For these reasons the measurement of energy balance can be important for studies of primate ecology, social structure, behaviour and reproductive ecology (Koenig *et al.*, 1997; Emery Thompson *et al.*, 2009). Inter-population variation in energetic status can also have a major impact on population viability. Population size is limited by the amount of food energy available to its members (the habitat's carrying capacity), so decreases in the availability of food can lead to reduced fecundity and increased mortality resulting in decreased population size (Altmann, 1974; Lee and Hauser, 1998; Brown, 2004; Raichlen *et al*., 2011). This has obvious implications for conservation, for example in predicting the impacts of climate change and habitat destruction on primate energy balance or in assessing the energy balance and therefore the vulnerability of already endangered populations (Cowlishaw and Dunbar, 2000).

Despite the importance of measuring primate energy balance, detailed estimations of energy intake and expenditure, which are required to determine energy balance, are limited (energy intake calculated: Vogel, 2005; Conklin-Brittain *et al*., 2006; Emery Thompson and Knot, 2008; Rothman *et al*., 2008; energy intake and expenditure calculated: Knott, 1998; Wasserman and Chapman, 2003; N'guessan *et al.*, 2009). This

situation is typified by baboon studies: despite many investigations into the baboon diet, few provide nutritional analysis of baboon foods (Whiten *et al*., 1991; Barton and Whiten, 1993; Barton and Whiten, 1994; Altmann, 1998) and calculation of the energetic intake of individual baboons has been carried out at just one study site (Stacey, 1986; Altmann, 1998; Muruthi *et al.*, 1991).

3.1.2.2. Weather data and Fruit Indices as proxies for food availability

Two categories of food availability proxy are used in this chapter. The first category is comprised of the weather data, which are expected to correlate with baboon food availability via their effect on primary productivity, since the majority of the baboon diet consists of plant items. Rainfall is tightly linked with primary productivity in the tropics (e.g. Boisvenue and Running, 2006; Toledo *et al.*, 2011) and is therefore predicted to correlate positively with energy balance. A positive relationship between temperature and primary productivity in dry and humid tropical forests has also been demonstrated (Toledo *et al.* 2011) and consequently a positive relationship between temperature and energy balance is also predicted. Both monthly and daily measures of rainfall and temperature are used here. Monthly values are used because primary productivity is more likely to be related to long term variability in weather patterns than day to day variations and daily values are included in order to examine whether these day to day variations directly affect the baboons' behaviour.

The second category of food availability proxy used here is comprised of the three fruit availability indices: vine-, tree-, and total-fruit index (section 2.1.2). These indices are expected to correlate positively with energy balance for three reasons: fruit is a major component of the baboon's diet; the baboons eat fruit belonging to a wide variety of species; and fruit tend to be among the most energetically valuable of the baboon's

foods. Although these data were collected only from within Kwano troop's, and not Gamgam troop's, home range they are used here alongside the energetic data from both troops because the two ranges share many of the same species and these are likely to fruit at around the same time, due to the closeness and climatic similarity of the two sites.

3.1.3. Predictions

In this chapter, data on the activity budgets and the calculated energy intake, expenditure and balance (together termed the calculated energetic status measures) of the Gashaka baboons are presented. The effects of weather, food availability, season, troop, rank and reproductive state on these measures are also examined. The following predictions were developed from the three study hypotheses laid out in section 1.4.2:

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

Prediction 1a: Activity budgets will differ between the two troops, reflecting the energetic benefits of Gamgam troop's crop-raiding. Relative to Kwano troop Gamgam troop will spend:

i. Less time feeding
ii. More time resting
iii. Less time travelling
iv. More time in social behaviours

Prediction 1b: Calculated energy balance of Gamgam troop will be higher than Kwano troop due to increased energy intake and/or decreased energy expenditure.

These predictions are based on the assumption that an examination of the difference between the activity budgets and energetic status measures of the two study troops is equivalent to an examination of the effects of food-enhancement. This assumption is made because the major difference between the troops is that one troop enhances its wild diet with raids from crop-fields and the other is entirely wild-feeding. Otherwise, the ecological pressures on the two troops are similar due to the fact that they are part of the same population and experience similar climate and habitat due to their geographical proximity. However, other differences do exist between the two troops such as a difference in habitat structure, with larger areas of closed forest in Kwano troop's range, and a difference in troop size, factors which can have important implications for feeding, travelling and social behaviour (e.g. Dunbar, 1992). It is therefore important to interpret the results of these analyses with caution since the effect of food-enhancement and other environmental factors cannot be easily disentangled when examining only two troops.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary

Prediction 2a: Activity budgets will differ between animals within troops reflecting their relative energetic costs and social status:

i. Pregnant and lactating animals will spend more time feeding or resting or less time travelling than cycling females

ii. Low ranking animals will spend more time feeding and less time resting than higher ranking females

Prediction 2b: Energetic status measures will differ between animals within troops:

i. Pregnant and lactating females will either have lower calculated energy balance than cycling females or increase their energy intake/ decrease their energy expenditure in order to maintain energy balance.

ii. Low ranking animals will have lower calculated energy balance (lower energy intake and/ or higher energy expenditure) than higher ranking animals.

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability.

Prediction 3a: Activity budgets will differ between the wet and dry seasons. During the wet season, when more food is available, the baboons will spend:

i. Less time feeding
ii. Less time travelling
iii. More time resting
iv. More time in social behaviours

Prediction 3b: Energetic status measures correlate with the weather variables in the following ways:

i. Calculated energy balance and intake will correlate positively with rainfall, temperature and the fruit indices
ii. Calculated energy expenditure will correlate negatively with rainfall, temperature and the fruit indices

3.2. Results

3.2.1. Activity budgets

3.2.1.1. Overview of activity budget data

Activity budgets were created using the focal follow data from 178 days consisting of between 2 and 8 hours of focal observation. As discussed in section 2.4.1, activity budgets were based on the percentage of total insight time that focal animals spent resting, travelling, feeding and in social behaviours. Periods of 'out of sight' time occurred on most observation days and consisted of times when the observer lost sight

of the focal animal, usually either during collection of urine or faecal samples or because the animal ran away or entered an area of vegetation in which the observer could not see the animal clearly and/or could not move through as quickly as a baboon. These incidences could also result in an observation day being terminated early, if the focal animal could not be found again, which resulted in some very short focal follows. In general visual contact was more often lost, and for greater periods, with Gamgam focals than with Kwano focals and this seemed to be associated with their crop-raiding behaviour (pers. obs.). The percentage of total observation time spent with the focal animal out of sight (%OS) was significantly greater for Gamgam troop than it was for Kwano troop (Mann-Whitney U test, U=3062, p=0.01). On average (median) across all seasons, Gamgam focal animals spent c. 12% of the observation day out of sight whereas Kwano focal animals spent c. 9% of the day out of sight (table 3.1).

Table 3.1. Median % of focal day spent with the focal animals Out of Sight across both troops and all seasons

	Median % of focal day spent OS (n)		
	Kwano	Gamgam	Both Troops
Dry	7.10(37)	10.10(30)	9.27(67)
Wet	9.31(57)	13.14(54)	11.76(111)
Whole year	8.52(n=94)	11.84(84)	10.78(178)

Focal days with less than 3 hours of in-sight time or where out-of-sight time accounted for more than 30% of total observation time were excluded from all further analyses since they were deemed unlikely to represent the focal animal's actual activity budget for that day. The remaining dataset consisted of 130 focal days, 68 for Kwano troop and 62 for Gamgam troop.

The four activity states used in these analyses together accounted for 99.89% of all in-sight time across both troops (Table 3.2). When all the data are considered together, the

two troops of Gashaka baboons spent around a third of their time feeding, a quarter to a third of their time resting and travelling and less than 10% in social behaviours (table 3.2).

Table 3.2. Total number of hours and mean percentage of in-sight time per day spent in specific activity states by focal animals from both troops

	Total hours spent in activity			**% of insight time spent in activity (mean±s.e.)**		
	Kwano (n=68)	**Gamgam (n=62)**	**Both troops (n=130)**	**Kwano (n=68)**	**Gamgam (n=62)**	**Both troops (n=130)**
Rest	98.44	118.25	216.69	25.81±1.35	35.74±1.74	30.55±1.17
Travel	104.38	87.45	191.83	27.08±0.90	26.26±0.91	26.69±0.64
Feeding	147.58	99.53	247.11	37.89±1.46	29.58±1.74	33.93±1.18
Social	35.43	27.55	62.98	9.15±0.91	8.26±0.81	8.73±0.61
All identified activities	385.83	332.78	718.61	99.94±0.02	99.85±0.02	99.89±0.01
Other	0.27	0.51	0.78	0.06±0.02	0.15±0.02	0.11±0.01
Total	386.1	333.29	719.39			

Effect of focal start time

When both troops are considered together, focal follows with early starts, as opposed to late starts, account for 55% of all follows. Within individuals the proportion of focals with early starts ranges from 30-100% (Table 3.3). However, no significant difference was found between early start and late start focals in terms of percentage of time spent resting (2-tailed t-test, t=0.042, d.f.=128, p=0.967), feeding (t=-0.088, p=0.930), travelling (t=-1.500, p=0.136) or in social behaviours (t=1.697, p=0.092). This suggests that the changes in the Gashaka baboon's activities throughout the day are not great enough for this bias in focal start time to affect the results of these analyses.

Table 3.3 Distribution of focal follows between early and late starts for each focal animal and both troops (continued over page).

	Number of focal follows:			**% focal follows:**	
ID	**Early start**	**Late start**	**Total**	**Early start**	**Late start**
BRA	1	0	1	100	0
DRK	2	4	6	33.33	66.67

Table 3.3. Continued from previous page.

	Number of focal follows:			% focal follows:	
FDI	3	3	6	50.00	50.00
KRM	3	0	3	100	0
KYE	6	3	9	66.67	33.33
LDI	4	3	7	57.14	42.86
LMI	4	2	6	66.67	22.22
MOM	7	1	8	87.50	12.50
SDY	6	2	8	75.00	25.00
TOJ	3	3	6	50.00	50.00
YMK	2	6	8	25.00	75.00
Kwano	**41**	**27**	**68**	**60.29**	**39.71**
BUD	6	5	11	54.55	45.45
KNE	6	6	12	50.00	50.00
MMK	8	5	13	61.54	38.46
MMW	3	7	10	30.00	70.00
STR	11	5	16	68.75	31.25
Gamgam	**34**	**28**	**62**	**54.84**	**45.16**
Both troops	**75**	**55**	**130**	**57.69**	**42.31**

3.2.1.2. Effect of troop, rank and reproductive success on activity budgets

The variation in activity budgets between different troops, ranks, reproductive states and seasons (table 3.4. and figures 3.1-3.4) was investigated using Generalised Linear Mixed Models (GLMMs). The factors were entered, as fixed effects, one at a time into 2-level models of the different activity states: rest, travel, feed and social behaviour. The dependent variable for each of the activity state models was the percentage of total in-sight time spent in that activity state. Focal follow number and focal ID were included as random effects to control for non-independence of multiple data points from the same individual (Section 2.4.3). Full results of statistical analyses relating to these GLMMs are given in appendix 6a, table A6.i, only the results of significant statistical analyses are presented here.

Table 3.4. Description of categorical explanatory factors featuring in the activity state GLMMs

Factor	Number of categories	Categories (number of data points)
Season	2	Dry (45), Wet (85)
Troop	2	Kwano (68), Gamgam (62)
Rank	3	High (53), Medium(33), Low (44)
Reproductive State	3	Cycling (48), Pregnant (19), Lactating (63)

Effect of Troop

The addition of 'troop' to the activity state 2-factor null models resulted in a significant increase in their explanatory power only for the resting and feeding models (likelihood ratio test, rest: D=9.172, d.f.=1, p=0.002; feeding: D=3.53, d.f.=1, p=0.002). Gamgam troop animals spent a significantly greater percentage of their time resting (z=3.55, p<0.001) and significantly less time feeding (z=3.63, p<0.001) than Kwano animals (figure 3.1). Percentage of time spent travelling and in social behaviours did not differ significantly between the two troops (figure 3.1).

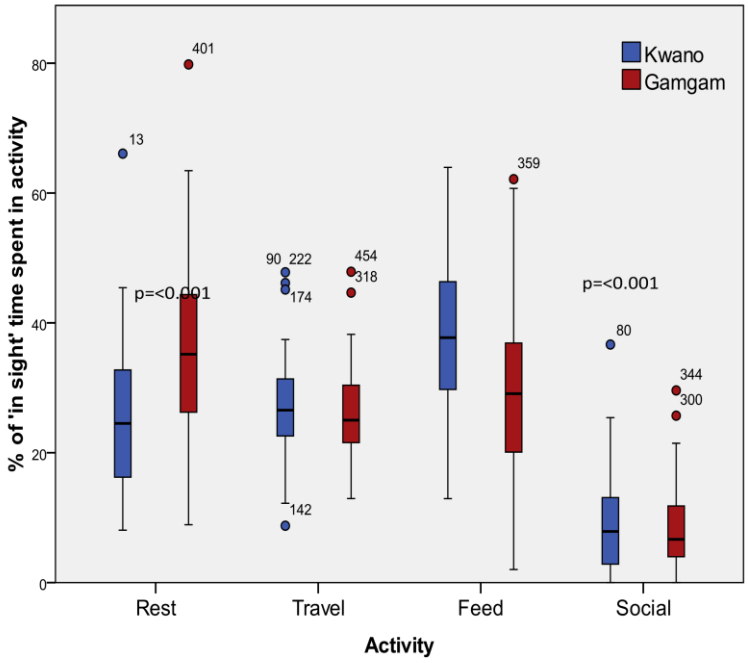

Figure 3.1. Box-plot showing percentage of time focal animals spent in the four main activity states for Kwano and Gamgam troops.

Effect of Season

The addition of 'season' to all of the activity state 2-factor null models resulted in a significant increase in their explanatory power (rest: D=16.68, p<0.001; travel: D=7.14, p=0.008; feed: D=12.51, p<0.001; social: D=5.76, p=0.016; d.f.=1 for all models). During the wet season animals spent a significantly greater percentage of their time resting (z=4.22, p<0.001) and in social behaviours (z=2.45, p=0.014) and significantly less time travelling (z=2.71, p=0.007) and feeding (z=3.63, p<0.001) compared to during the dry season (table 3.6, figure 3.2).

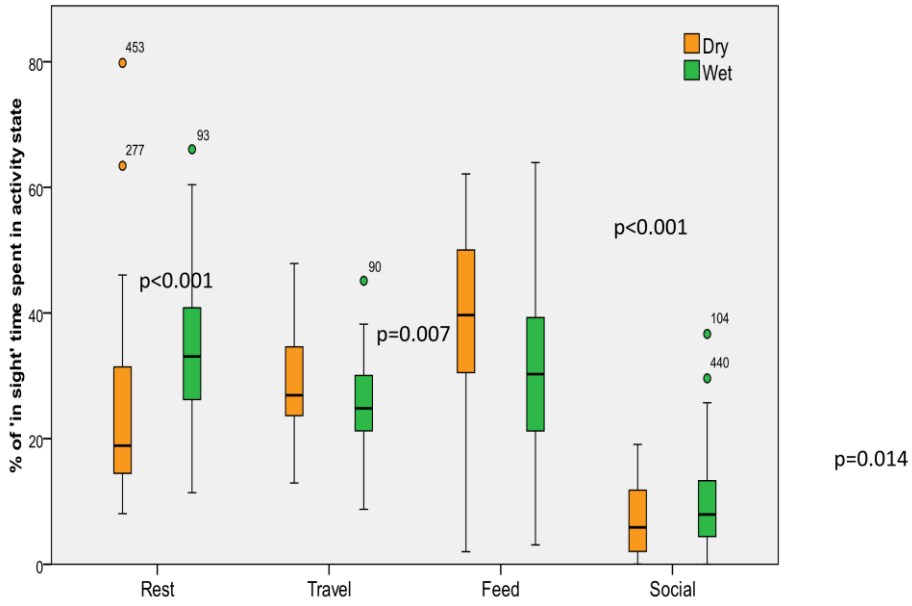

Figure 3.2. Box-plot showing percentage of time focal animals spent in the four main activity states during the dry and wet seasons.

Effect of Rank

The addition of 'rank' did not significantly increase the explanatory power of any of the activity state 2-level null models. Animals of different ranks did not differ significantly in the percentage of time they devoted to the four main activity states (appendix 6a, table A6.i).

Effect of Reproductive State

The addition of 'reproductive state' did not significantly increase the explanatory power of any of the activity state 2-level null models. Animals in different reproductive state did not differ significantly in the percentage of time they devoted to the four main activity states (appendix 6a, table A6.i).

Interaction effects

All possible 2-way interaction effects between the categorical variables (troop, season, rank and reproductive state) were entered into the activity state models to allow for the possibility that the effects of each category differed between individuals of different types e.g. the effect of season on resting behaviour may differ between troops. No more than 2 categorical variables where added into the model at a time (e.g. the effect of the potential 3-way interaction between troop, rank and season was not tested) since the addition of a third categorical variable meant that the number of data points, and the number of individuals contributing to those data points, within each category would be too small for meaningful analyses.

The troop-rank interaction effect resulted in a significant increase in the explanatory power of the travel ($D=6.14$, d.f.=2, $p=0.046$) and social ($D=6.42$, d.f.=2, $p=0.040$) models only. Middle and low ranking animals from Kwano troop spent a greater percentage of their time travelling than high ranking animals, although in both cases the difference was marginally non-significant (middle>high: $z=3.64$, $p=0.070$; low>high: $z=1.67$, $p=0.096$), and no effect of rank on travelling time was detected for Gamgam troop (figure 3.3).

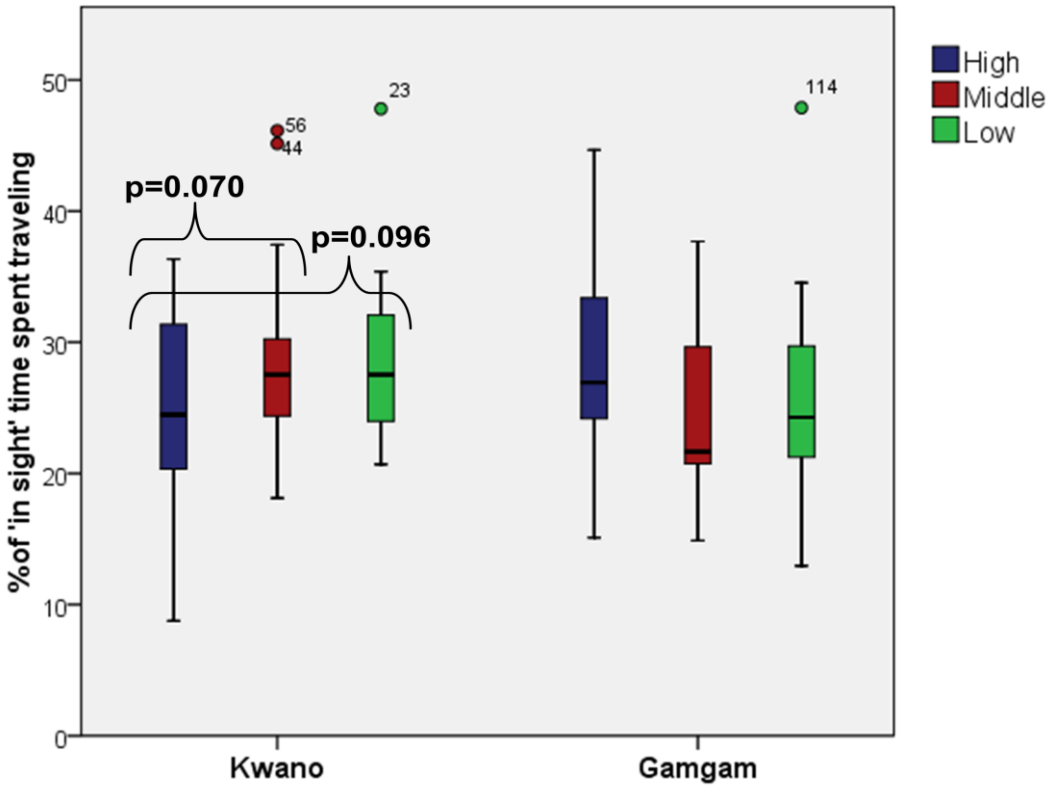

Figure 3.3. Box-plot showing percentage of time spent travelling by focal animals of different ranks and troops.

High and low ranking animals from Kwano troop spent a greater percentage of their time in social behaviours than middle ranking animals, although in both cases the difference was marginally non-significant (high>middle: z=1.82, p=0.07; low>middle: z=1.89, p=0.058). Within Gamgam troop, the one middle ranking animal spent a significantly greater percentage of her time in social behaviours than the two high ranking animals (z=2.30, p=0.022) (figure 3.4).

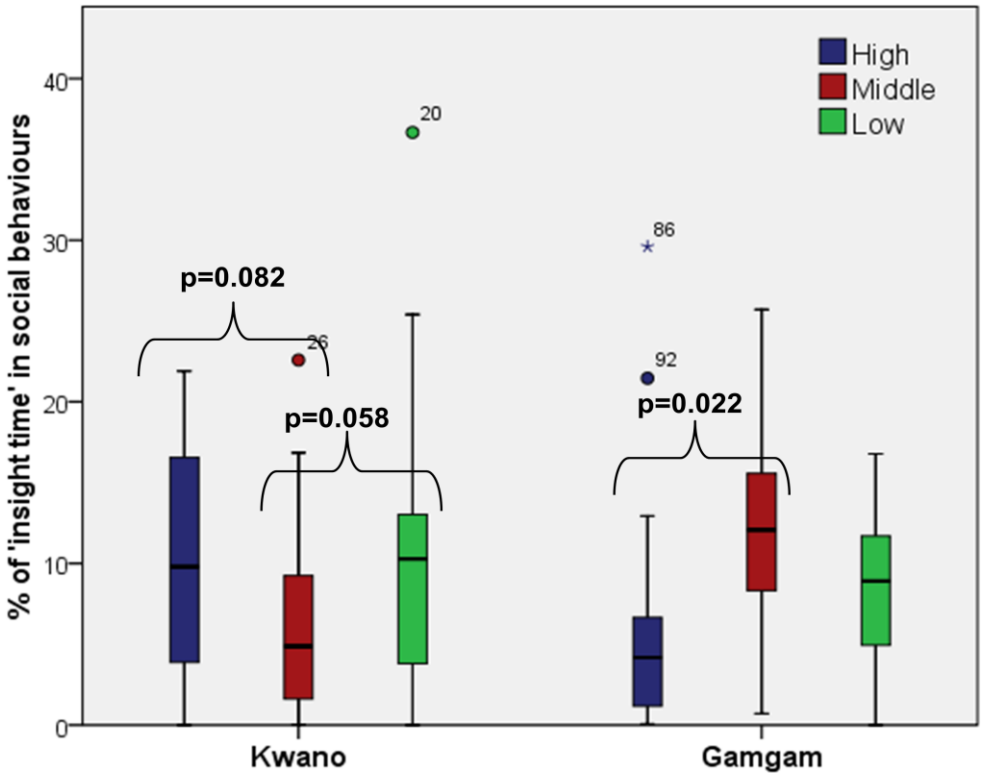

Figure 3.4. Box-plot showing percentage of time spent in social behaviours by focal animals of different ranks and troops.

Effect of Gamgam troop's crop-raiding behaviour

In order to examine the direct effect of crop-raiding behaviour on the activity budgets of the Gamgam troop members a comparison was made between days when a crop species was observed being eaten by the focal animal (n=11) and those days when no crop food items were observed being eaten (n=51). There was no significant difference in the proportion of time spent in any of the four main activity budget components between crop-feeding and non-crop-feeding days (independent sample t-tests, d.f.=60 for all. Rest: t=-0.26, p=0.794; travel: t=-0.62, p=0.535; feed: t=1.24, p=0.220; social: -1.37, p=0.177).

3.2.1.3. Comparison of 2009 Gashaka activity budgets with data from 2001/2 and other sites

As discussed in section 1.3.2 Kwano and Gamgam troops were first habituated and studied by Ymke Warren between 2000 and 2002. As part of this study Warren determined the activity budgets of the troops by performing scan samples of all troop members' activities every 2.5 minutes. Warren discussed her results in relation to similar activity budget data from other baboon populations across Africa (Warren, 2003), particularly in reference to studies which had compared food-enhanced and wild-feeding troops. Here, the activity budget data from the present study (2009) are compared to those obtained by Warren between May 2001 to April 2002, and Warren's comparison of the Gashaka population with other study populations will be briefly repeated with the present data.

Apart from the inevitable differences in the data resulting from the fact that this study took place several years after Warren's (e.g. yearly variations in weather, change in troops' sizes and compositions) there are several differences in the method of data collection which make direct comparisons more difficult. In the current study, the method of data collection was continuous focal sampling and only adult females were sampled (n=16). In contrast, Warren (2003) used the method of scan sampling and the behaviours of all group members, including adult males, juveniles and infants were recorded. This difference is very likely to have an effect on a troop's average activity budget since primate nutritional requirements and therefore activity budgets vary with sex and age (section 1.12.). Another difference lies in the categorisation of cheek-pouch behaviour. For the creation of activity budgets, Warren included cheek-pouch in with her 'foraging' activity category whereas in the current study cheek-pouch has been grouped together with resting behaviours. When comparing the activity budgets from

the current study with Warren's data, cheek-pouch behaviour is therefore included within the category 'feed' rather than within the category 'rest'. For comparison with data from other studies, and for all other analyses, cheek-pouch is included within the resting category.

The percentage of time Kwano and Gamgam troops spent in the four main activity states was compared using independent t-tests for both the 2001/2 and 2009 data sets (Table 3.5). For the purpose of this comparison, cheek-pouch behaviour is included within the category 'feed'.

Table 3.5. Activity budgets and the results of independent t-tests between the daily activity budgets of Kwano and Gamgam troops from 2001/2 and 2009

	2001/2 (d.f.=152)			2009 (d.f.=128)			
	Kwano	**Gamgam**	**p**	**Kwano**	**Gamgam**	**t**	**P**
Rest	27.8	34.5	< **0.001**	18.62	28.24	-4.830	<**0.001**
Travel	29.6	25.2	**0.001**	27.08	28.24	0.644	0.521
Social	8.5	11	**0.004**	9.15	8.26	0.720	0.473
Feed	31.2	26.7	**0.012**	45.09	37.08	3.547	**0.001**

- 2001/2 data collected by Y. Warren May-Aug 2001 and Nov 2001-April 2002 (table adapted from Warren, 2003)
- 2009 data are from the current study

In both studies focal animals from Kwano troop spent a significantly greater percentage of their time feeding and significantly less time resting than those from Gamgam. During 2001/2 Kwano troop focal animals also spent a significantly greater percentage of their time travelling and significantly less time in social behaviours. In contrast, there is no significant difference in the percentage of time members of Kwano and Gamgam troops devote to travelling and social behaviours in the 2009 data set (table 3.2).

The percentage of observation time spent feeding was substantially higher in the 2009 dataset compared to the 2001/2 dataset for both troops whereas the percentage of time

the animals spent resting was substantially lower in the 2009 dataset compared to the 2001/2 dataset. Travel and social time were fairly similar in both studies (table 3.5, figure 3.5).

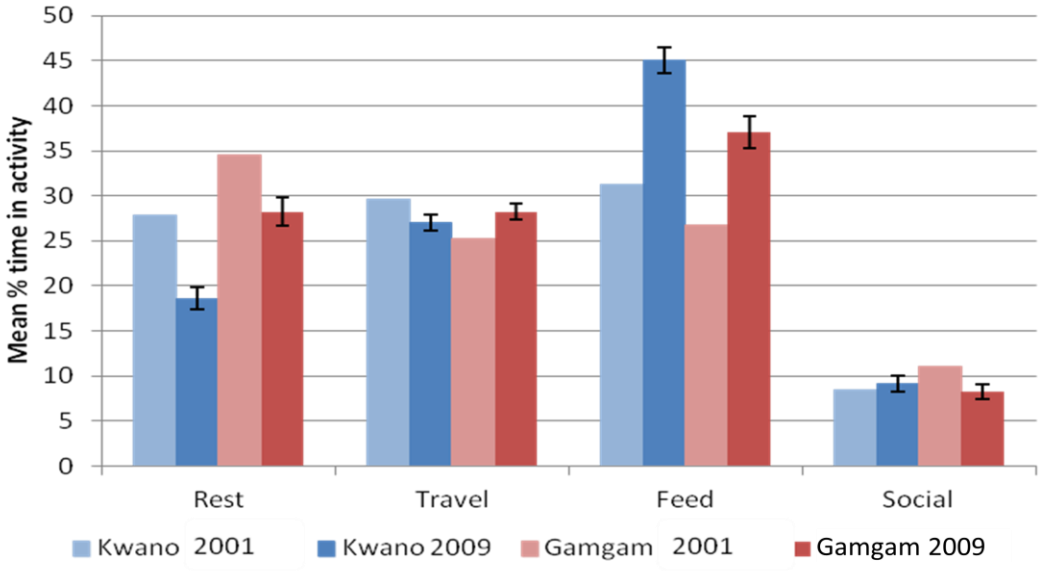

Figure 3.5. Activity budgets from 2001/2 and 2009 data for both troops. 2001/2 bars represent overall activity budget and 2009 bars represent mean daily activity budget with error bars representing the standard error. Cheek pouch behaviour included within 'feed' category.

Warren (2003) also examined the interaction between troop and season using independent t-tests. Equivalent analyses with the 2009 data were carried out and results are presented in table 3.6 alongside the 2001/2 relationships.

Table 3.6. Variation in 2009 activity budgets between the Kwano and Gamgam troops during the wet and dry seasons and the results of t-tests comparing daily activity budgets between seasons within troops and between troops within seasons. Results of equivalent analyses performed on the 2001/2 activity budgets are included for comparison (continued over page)

	Mean % of in-sight time		**t**	**d.f.**	**p**	**2009 relationship**	**2001/2 relationship**
Kwano	**Dry**	**Wet**					
Rest	14.83	20.42	-2.118	66	**0.038**	**W > D**[2]	**W > D**
Travel	30.14	25.62	2.438	66	**0.017**	**D > W**	**D > W**
Feed[1]	47.06	44.14	.943	66	0.349	N.S.	***D > W***
Social	7.92	9.74	-.932	66	0.355	N.S.	N.S.

Table 3.6. Continued from previous page

	Mean % of in-sight time		t	d.f.	p	2009 relationship	2001/2 relationship
Gamgam	**Dry**	**Wet**					
Rest	25.02	30.14	-1.611	60	0.113	N.S.	**W > D**
Travel	27.89	25.30	1.393	60	0.169	N.S.	**D > W**
Feed	40.50	35.06	1.512	60	0.136	N.S.	N.S.
Social	6.37	9.38	-1.818	60	***0.074***	***W > D****	N.S.
Dry	**Kwano**	**Gamgam**					
Rest	14.83	25.02	-3.131	36.1	**0.003**	**G > K**	**G > K**
Travel	30.14	27.89	.938	43	0.354	N.S.	**K > G**
Feed	47.06	40.50	1.799	43	***0.079***	***K > G***	N.S.
Social	7.92	6.37	.915	37.2	0.366	N.S.*	**G > K**
Wet	**Kwano**	**Gamgam**					
Rest	20.42	30.14	-3.986	83	**<0.001**	**G < K**	**G < K**
Travel	25.62	25.30	.227	83	0.821	N.S.	N.S.
Feed	44.14	35.06	3.192	83	**0.002**	**K > G**	N.S.
Social	9.74	9.38	.217	83	0.829	N.S.	N.S.

1. Cheek-pouch behaviour is included within the 'feed' category for 2009 and 2001/2 data
2. **Bold** indicates that difference is significant at p<0.05, ***bold and italics*** indicates a trend, p<0.1

* Instances where the 2009 relationship is in the opposite direction to that found in the 2001/2 data

There is general agreement between the 2001/2 and 2009 results in terms of how troop and season affect activity budgets, and what differences there are tend to be due to whether or not a trend is significant rather than a difference in the direction of relationship. A change in the direction of the relationship only occurred in two cases, both concerning social behaviour and neither significant (Gamagam social time > Kwano during the dry season in 2001/2 but < Kwano in 2009 and dry season social time > wet season for Gamgam in 2001/2 but < wet season in 2009).

The 2009 activity budgets were compared with similar data from other study sites with wild-feeding and food-enhanced baboons, following Warren (2003). The summary data from table 5.4 in Warren (2003) is reproduced below in table 3.7 giving the mean proportion of time wild-feeding and food-enhanced baboon troops from different populations spent in the four activity states alongside the Gashaka data from 2001/2 and 2009 (see Warren 2003 for references). For these comparisons, cheek-pouch behaviour

is not included within the category ‘feed’ for the 2009 data, consistent with studies from other populations (Forthman-Quick 1986; Forthman-Quick and Demment, 1988) and instead is included within the ‘rest’ category.

Table 3.7. Summary of activity budget data from wild-feeding and food-enhanced baboons. 2001/2 and 2009 Kwano and Gamgam activity budgets are presented alongside average activity budgets for wild-feeding and food-enhanced baboons calculated from data collected at study sites other than Gashaka (adapted from table 5.4 in Warren 2003, see references therein).

	Feed	**Rest**	**Social**	**Travel**
	Wild-feeding			
Kwano 2001/2	31	28	9	30
Kwano 2009	38	26	9	27
Data from other sites	**n=22**			
Mean	40	25	12	26
Standard deviation	12	13	5	7
Minimum	20	6	5	9
Maximum	65	62	22	37
	Food-enhanced			
Gamgam 2001/2	27	35	11	25
Gamgam 2009	30	36	8	27
Data from other sites	**n = 7**	**n = 5**	**n = 5**	**n = 4**
Mean	26	33	11	24
Standard deviation	10	9	3	3
Minimum	11	22	7	19
Maximum	48	44	16	27
Mean difference between wild-feeding and food-enhanced troops				
Gashaka 2001/2	-4	+7	+2	-5
Gashaka 2009	-8	+10	-1	0
Other studies	-14	+8	-1	-2

The Gashaka baboon activity budgets from both 2001/2 and 2009 fall within the range of other baboon studies. The wild-feeding (Kwano) activity budgets from both the Gashaka studies are more similar to the average from the other field sites than are the food-enhanced (Gamgam) activity budgets. For Kwano the results of the current study lie nearer the average than those from the 2001/2 study, whereas for Gamgam, the results of the 2001/2 study are closer to the average.

A summary of within study site comparisons of the activity budgets of wild-feeding and food-enhanced baboons, reproduced from Warren (2003), is presented in table 3.8. alongside the Gashaka 2001/2 and 2009 results.

Table 3.8. Summary of within study differences in activity budgets between wild-feeding and food-enhanced baboon groups, comparing the results from (1) previous studies, (2) Gashaka baboons 2001/2 and (3) Gashaka baboons 2009. Percentages are of total observation time (table adapted from Warren 2003)

	1. Wild-feeding cf. Food-enhanced baboons **2. 2001/2 Kwano troop cf. Gamgam troop** **3. 2009 Kwano troop cf. Gamgam troop**
Foraging	1. Wild-feeding baboons spend 20-43% more time foraging than food-enhanced baboons 2. Kwano troop spent 4% more time feeding than Gamgam troop 3. Kwano troop spent 8% more time feeding than Gamgam troop
Rest	1. Wild-feeding baboons spend 17-36% less time resting than food-enhanced baboons 2. Kwano troop spent 7% less time resting than Gamgam troop 3. Kwano troop spent 10% less time resting than Gamgam troop
Social activity	1. In the first study at Gilgil, Forthman-Quick (1986) found that the baboons with minimal food-enhancement (0.7% of observations) spent 2% more time in social activity than the food-enhanced baboons. Other research has found wild-feeding baboons spend 4-10% less time in social activity than food-enhanced baboons 2. Kwano troop spent 2% less time in social activity than Gamgam troop 3. Kwano troop spent 1% less time in social activity than Gamgam troop
Travel	1. The Forthman-Quick (1986) study at Gilgil found that the baboons with minimal food-enhancement spent 3% less time in travel than the food-enhanced baboons. Other research has found wild foraging baboons spend 5-6% more time in travel than food-enhanced baboons 2. Kwano troop spent 4% more time in travel than Gamgam troop 3. Kwano troop spent 1% more time travelling than Gamgam troop

The effect of food-enhancement in reducing feeding time and increasing resting time is less for the Gashaka baboons than was found at other sites. The 2009 data are closer to the average food-enhancement effect for feeding and resting but the 2001/2 data are closer for social behaviours and travel.

3.2.2. Calculated energy measures

3.2.2.1. Overview of calculated energy balance data

For each focal day the observed energy intake and observed energy expenditure were calculated using the activity budget, nutritional analysis and daily travel distance data alongside published energy use conversion factors (see section 2.4.2). The energy expenditure value for each day was then subtracted from the energy intake to give a value of energy balance, in kJ, for each focal day (Table 3.9.)

Table 3.9. Summary of energy balance data for Kwano and Gamgam troops including minimum, maximum and mean values plus the number of focal days resulting in positive or negative energy balance values.

	Energy balance values in kJ:			Number of focal days:		
	Min	**Max**	**Mean ± s.e.**	**Total**	**In -ve energy balance**	**In +ve energy balance**
Kwano	-1896	25643	4255 ± 672	68	18	50
Gamgam	-2100	31280	7839 ± 935	62	9	53
Both troops			5964 ± 587	130	27	103

Energy balance scores were partitioned into three categories: energy debt, with an energy balance score less than -1000kJ; energy balance, with an energy balance score between -1000kJ and+1000kJ; and energy surplus, with an energy balance score greater than +1000kJ (table 3.10). Energy debt accounted for 10% of focal days, energy balance accounted for 19% of focal days and energy surplus accounted for 71% of focal days. The two troops experienced the same proportion of energy debt days but Kwano troop experienced more energy balance days and fewer energy surplus days than Gamgam. Three times as many energy debt days occurred during the dry season compared to the wet season and a third more energy surplus days occurred during the wet season compared to the dry season (table 3.10).

Table 3.10. Distribution of focal observation days with energy surplus, balance and debt between troops and seasons

	Dry	**Wet**	**Both seasons**
% of days in energy debt (< -1000kJ)			
Kwano	23	4	10
Gamgam	13	8	10
Both troops	**18**	**6**	**10**
% of days in energy balance (-1000 and +1000kJ)			
Kwano	23	28	26
Gamgam	26	3	11
Both troops	**24**	**16**	**19**
% of days in energy surplus (>+1000kJ)			
Kwano	55	67	63
Gamgam	61	90	79
Both troops	**58**	**78**	**71**

These results suggest that energy intake is being over estimated and/or energy expenditure is being underestimated, since free-living, healthy animals are generally expected to be in energy balance. Possible reasons for this are discussed in section 3.3.2, but for the purposes of this chapter energy intake and energy expenditure will be examined separately since an examination of energy balance would be likely to underplay the effect of energy expenditure and overplay the effect of energy intake. Daily values of calculated energy intake and expenditure per hour (energy intake and expenditure rate) are used as the units of analyses in this chapter to control for difference in the length of daily observation periods.

3.2.2.2. Determinants of energy intake rate

Weather and fruit index variables

The relationship between calculated energy intake rate and the weather and fruit index variables was assessed using GLMMs. Focal ID and focal day number were fitted into the models as random effects and the weather and fruit index variables were fitted into the model, one at a time, as fixed effects.

The addition of daily and monthly minimum and maximum temperatures (figure 3.6) and the three fruit indices (figure 3.7), but not daily or total monthly rainfall, significantly improved the explanatory power of the energy intake rate 2-factor null model. The relationship between calculated energy intake rate and these variables was significant and positive in all cases (table 3.11, coefficients only included if the model was significantly better than the 2-factor null model)

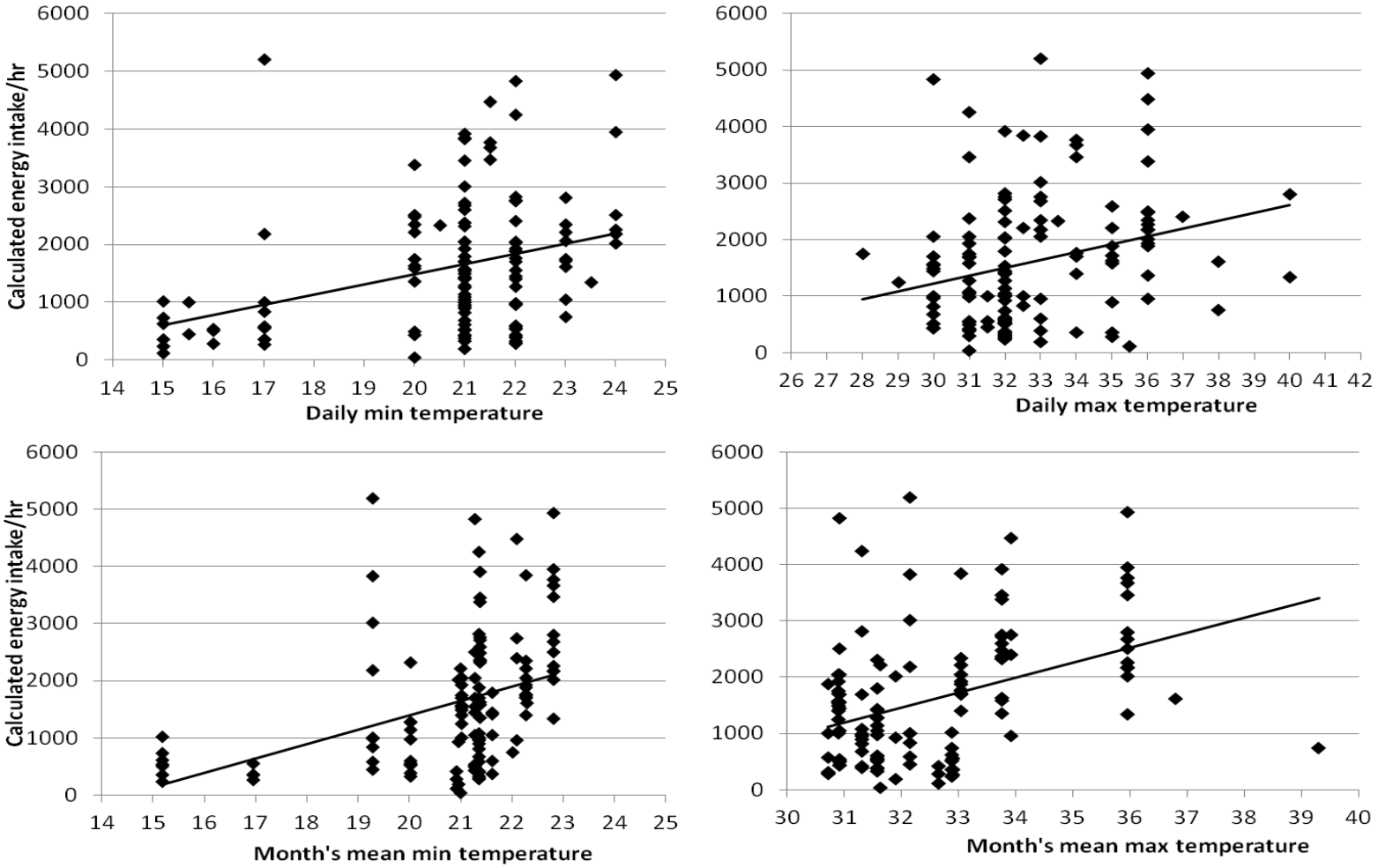

Figure 3.6. Scatter plots showing the relationship between calculated energy intake rate (kJ/hr) and four measures of temperature (^{o}C). The lines represent the average linear relationships between the two variables for all individuals, as predicted by the GLMM.

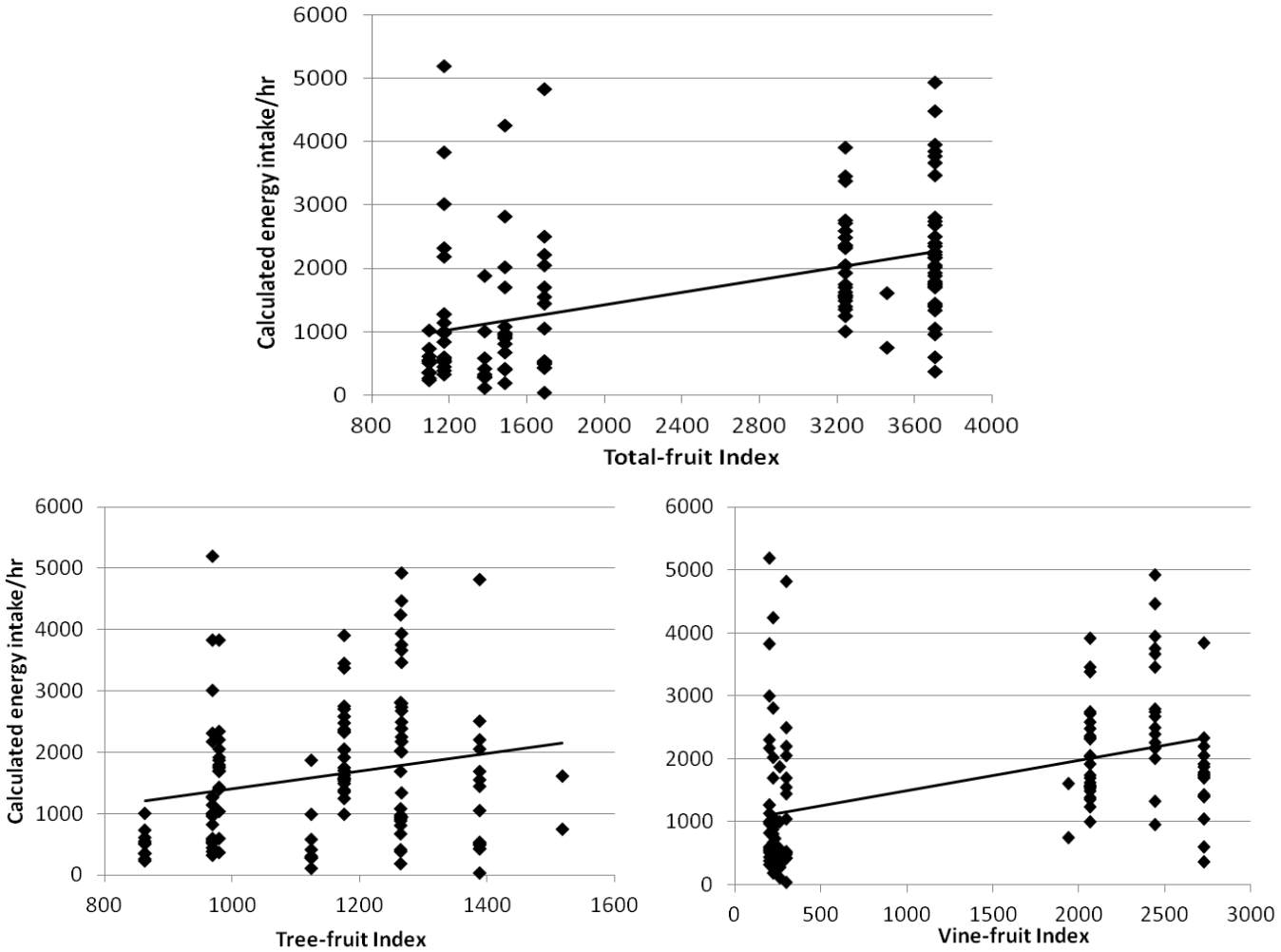

Figure 3.7 Scatter plots showing the relationship between calculated energy intake rate (kJ/hr) and the three fruit indices. The lines represent the average linear relationships between the two variables for all individuals, as predicted by the GLMM.

Table 3.11. Results of likelihood ratio tests and z tests from the GLMMs of the relationship of the weather and fruit index variables to calculated energy intake rate. Each row represents a separate model.

Explanatory factors	D	p	Coefficient	s.e.	z	p
Daily min temp	19.04	<**0.001**	188.13	40.99	4.59	<**0.001**
Daily max temp	7.89	**0.005**	137.94	43.92	3.14	**0.002**
Daily rainfall	0.69	0.408				
Mean monthly min temp	22.42	<**0.001**	251.30	50.69	4.96	<**0.001**
Mean monthly max temp	19.90	<**0.001**	265.93	54.97	4.84	<**0.001**
30-day rainfall	2.38	0.123				
Total-fruit index	30.52	<**0.001**	0.49	0.08	6.06	**0.009**
Vine-fruit index	7.52	**0.006**	1.62	0.57	2.83	**0.005**
Tree-fruit index	27.08	<**0.001**	0.48	0.08	5.680	<**0.001**

- D statistic relates to comparison with 2-factor null model
- d.f.=1 in all cases

Season

The addition of season to the energy intake rate 2-factor null model, as a categorical, fixed variable, did not result in a significant increase in the model's explanatory power (D=2.746, d.f.=1, p=0.097) but there was some sign of a trend towards higher energy intake rates during the wet season (z=1.67, p=0.095; figure 3.8).

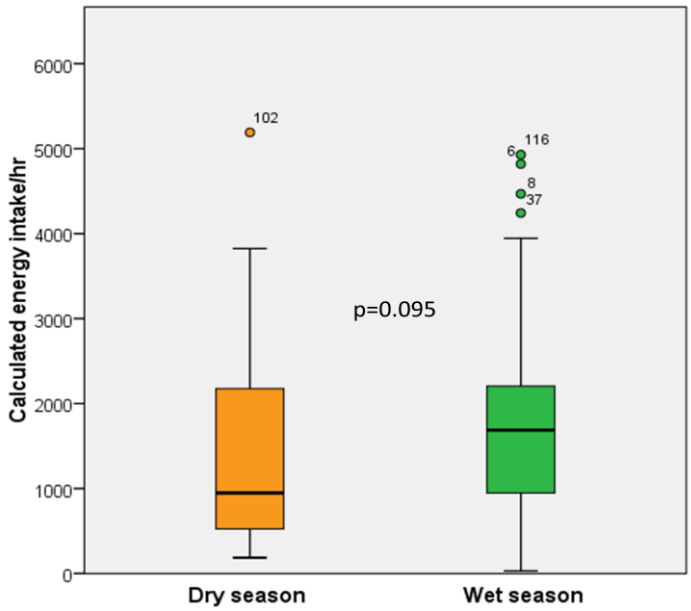

Figure 3.8. Box-plot showing calculated energy intake rates (kJ/hr) of focal animals during the dry and wet seasons.

The addition of the season interaction effect, to the weather and fruit index models significantly increased the explanatory power of three out of the nine models, which means that for these three models the relationship between the explanatory factor and energy intake rate differs between seasons (full results of statistical analyses in appendix 6a, table A6.ii). Out of those which correlated significantly with energy intake rate (the temperature measures and fruit indices) only monthly mean minimum temperature acquired a significant increase in its explanatory power with the addition of the season interaction ($D=4.53$, d.f.=1, $p=0.033$; figure 3.9). Although the relationship between energy intake rate and mean monthly minimum temperature is significant and positive within both seasons, the interaction effect is significant apparently due to the large difference in the range of monthly minimum temperatures within the two seasons. Within the dry season, mean monthly minimum temperatures ranged from 15-23^{o}C whereas in the wet season the range is between 21 and 23^{o}C. The slope of the relationship for the wet season data is therefore steeper than for the dry season, due to the far narrower range of mean minimum temperatures.

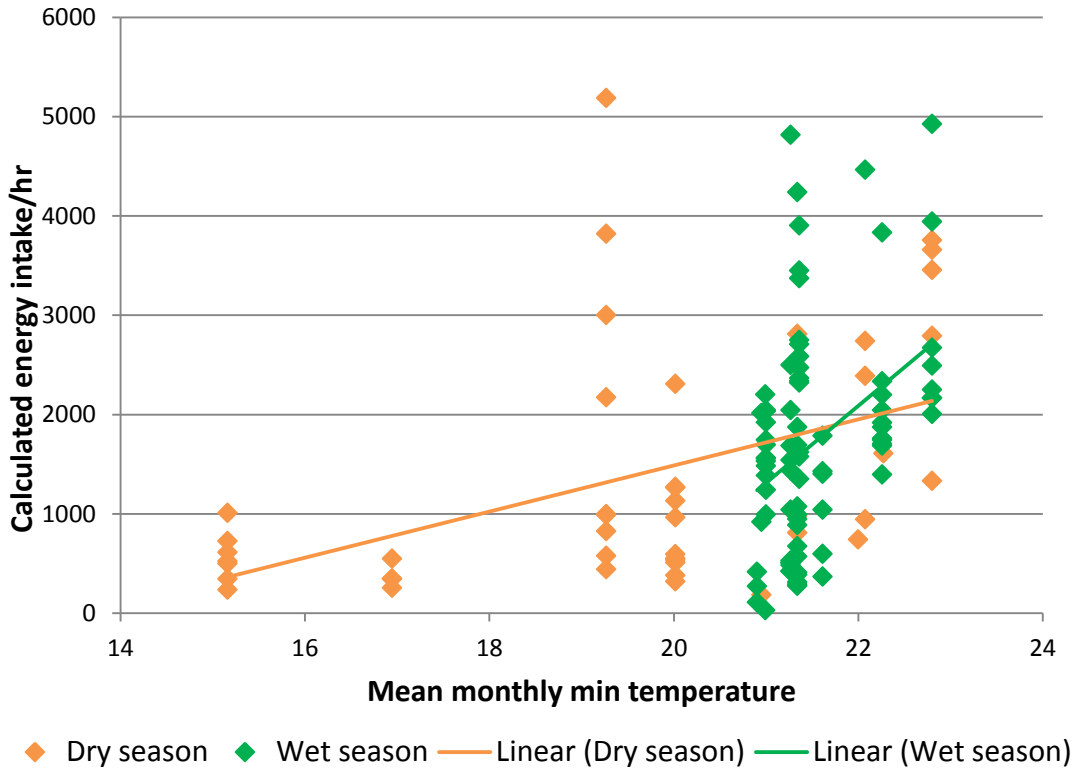

Figure 3.9. Scatter plot showing the effect of season on the relationship between calculated energy intake rate (kJ/hr) and mean monthly minimum temperature (^{o}C). The two lines represent the average linear relationships between the two variables for all individuals for each season, as predicted by the GLMM.

The addition of the season interaction effect also significantly increased the explanatory power of the models containing both daily ($D=4.63$, d.f.=1, $p=0.031$) and 30-day rainfall ($D=8.37$, d.f.=1, $p<0.001$). In both cases there was a significant and negative correlation with energy intake rate during wet season (daily rain: $z=2.13$, $p=0.033$; 30-day rain: $z=4.80$, $p<0.001$) but no significant relationship with energy intake rate during the dry season. There was even a trend towards a positive relationship between daily rainfall and energy intake rate ($z=1.83$, $p=0.068$, indicated by a dashed best fit line) (figure 3.10 a and b).

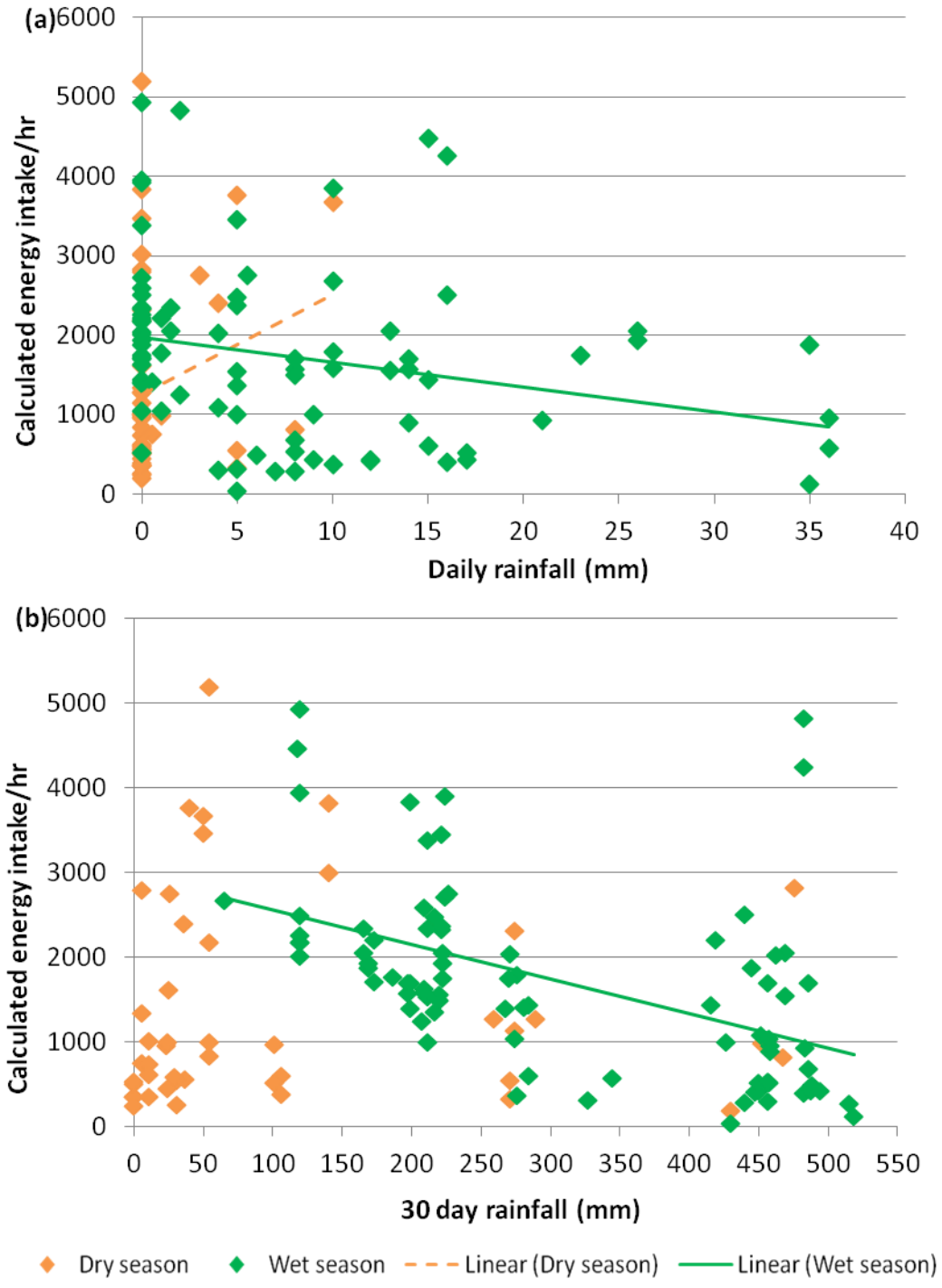

Figure 3.10. Scatter plots showing the effect of season on the relationship between calculated energy intake rate (kJ/hr) and (a) daily rainfall and (b) total monthly rainfall. The lines represent the average linear relationships between the two variables for all individuals for each season, as predicted by the GLMM.

Troop

The addition of troop to the 2-factor energy intake rate null model, as a categorical, fixed variable, resulted in a significant increase in the model's explanatory power

(D=10.79, d.f.=1, p=0.001). Gamgam animals exhibited significantly higher energy intake rate than Kwano animals (z=3.61, p<0.001; figure 3.11).

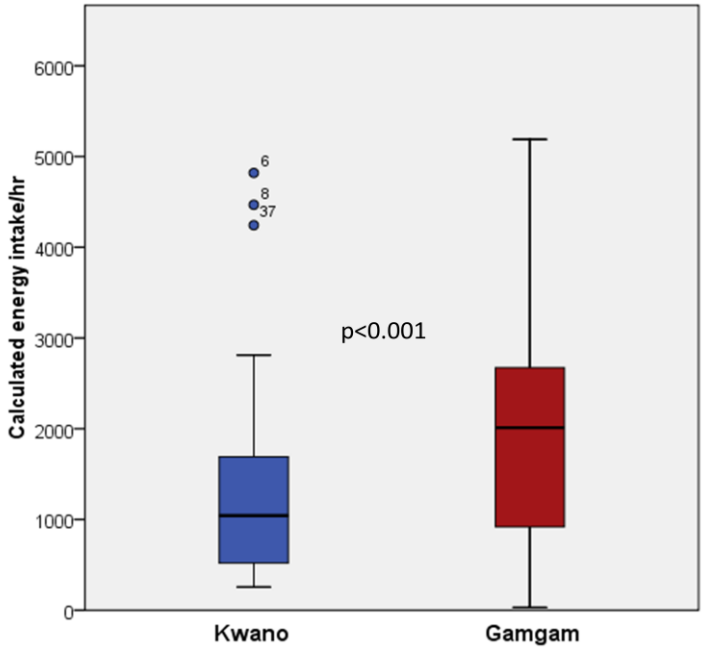

Figure 3.11 Box-plot showing the calculated energy intake rates (kJ/hr) of focal animals belonging to different troops.

The addition of the troop interaction effect did not significantly increase the explanatory power of any of energy intake rate models containing the weather measures and fruit indices, which means the relationships between energy intake rate and the weather measures or fruit indices were the same for both troops despite Gamgam troop generally experiencing higher energy intake rates (appendix 6a, table A6.ii). Examples of this effect are demonstrated in Figure 3.12 for (a) daily minimum temperature and (b) tree-fruit index.

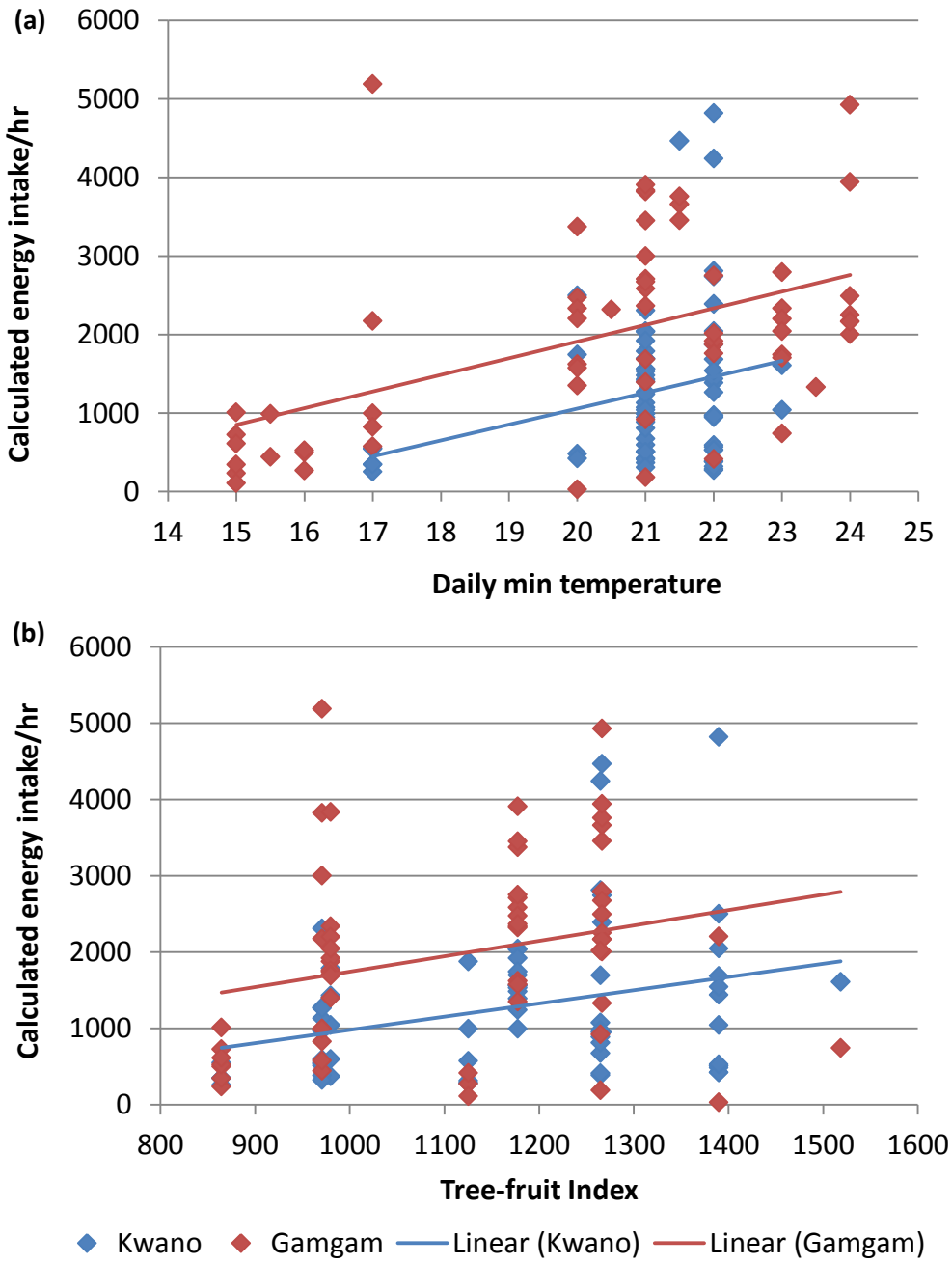

Figure 3.12 Scatter plots showing the effect of troop on the relationship between calculated energy intake rate (kJ/hr) and (a) daily minimum temperature (^{o}C) and (b) tree-fruit index. The lines represent the average linear relationships between the two variables for all individuals in each troop, as predicted by the GLMM.

Rank

The addition of rank to the 2-level energy intake rate null model, as a categorical, fixed variable, did not result in a significant increase in the model's explanatory power meaning that there was no significant difference between the calculated energy intake rates of individuals of different ranks (D=2.62, d.f.=2, p=0.270). The addition of the

rank interaction effect to the 2-level energy intake rate model containing mean monthly maximum temperature, significantly improved its explanatory power (D=8.12, d.f.=2, p=0.017). There was a significant and positive relationship between energy intake rate and mean monthly maximum temperature for high (z=2.93, p=0.003) and low (z=4.71, p<0.001) ranking animals but not for middle ranking animals (z=0.74, p=0.458) (figure 3.13). There was no significant effect of rank on any of the other energy intake rate relationships (appendix 6a, table A6.ii).

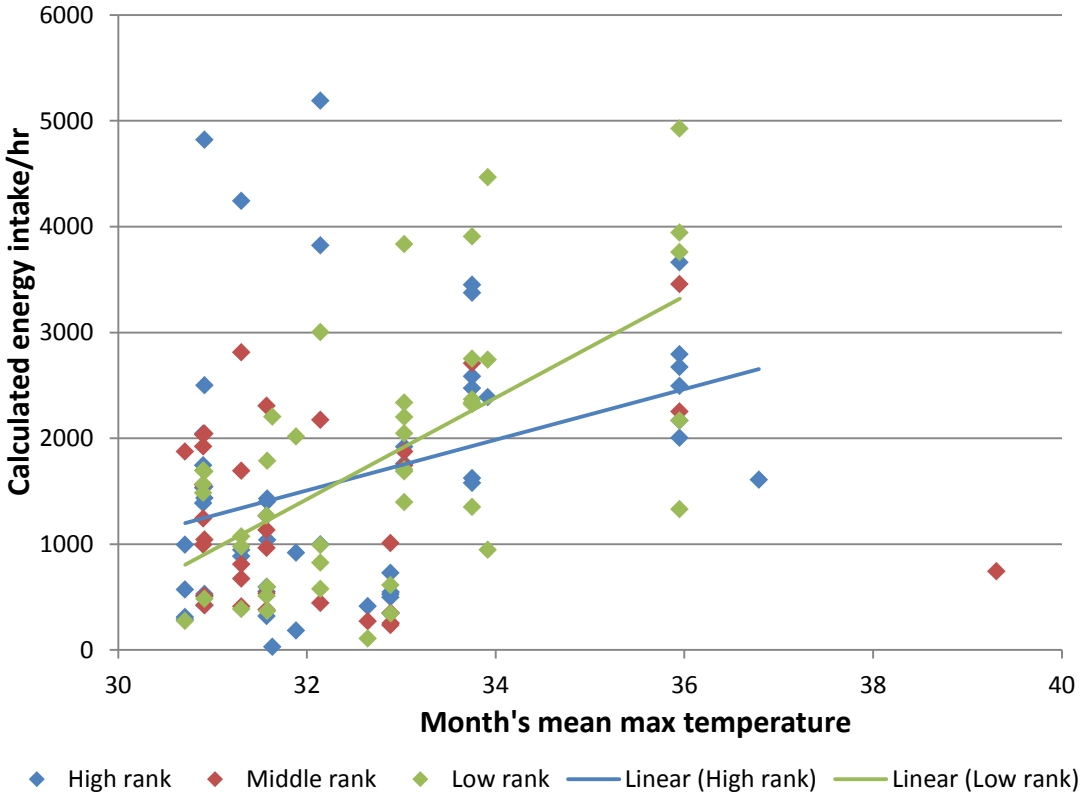

Figure 3.13. Scatter plots showing the effect of rank on the relationship between calculated energy intake rate (kJ/hr) and mean monthly maximum temperature (^{o}C). The lines represent the average linear relationships between the two variables for all individuals of high or low rank, as predicted by the GLMM.

Reproductive state

The addition of reproductive state to the 2-level energy intake rate null model, as a categorical, fixed variable, did not result in a significant increase in the model's explanatory power (D=4.29, d.f.=2, p=0.117). However, there was a tendency for pregnant animals to exhibit a higher energy intake rate than both cycling (z=1.90, p=0.057) and lactating (z=2.01, p=0.045) animals (figure 3.14).

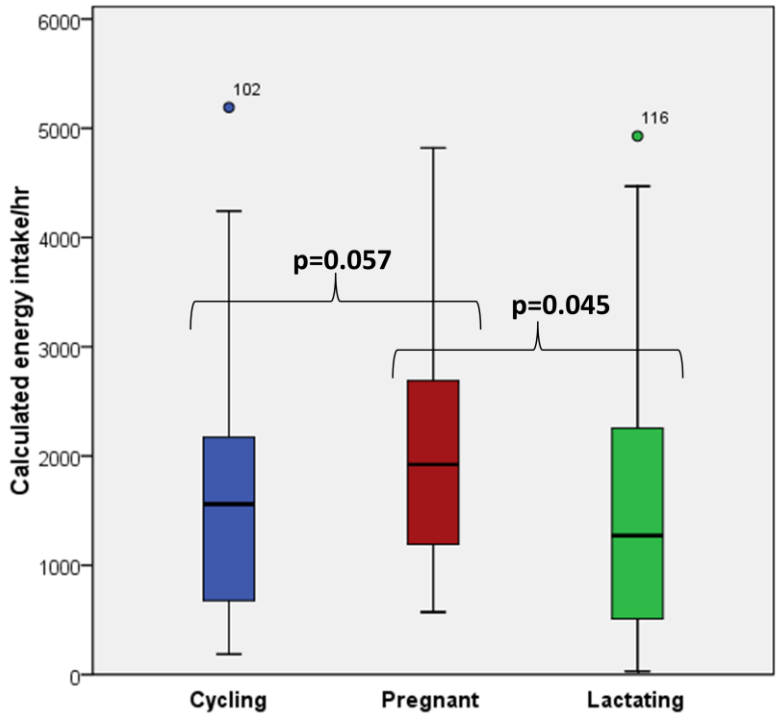

Figure 3.14. Box-plot showing the variation in calculated energy intake rate (kJ/hr) between focal animals in different reproductive states.

The addition of the reproductive state interaction effect to the 2-level energy intake rate model containing mean monthly maximum temperature significantly improved its explanatory power (D=10.13, d.f.=2, p=0.006). There was a significant and positive relationship between energy intake rate and mean monthly maximum temperature for lactating animals (z=5.69, p<0.001), a marginally non-significant positive relationship for pregnant animals (z=1.93, p=0.054) and no sign of a relationship for cycling animals (z=1.34, p=0.180) (figure 3.15).

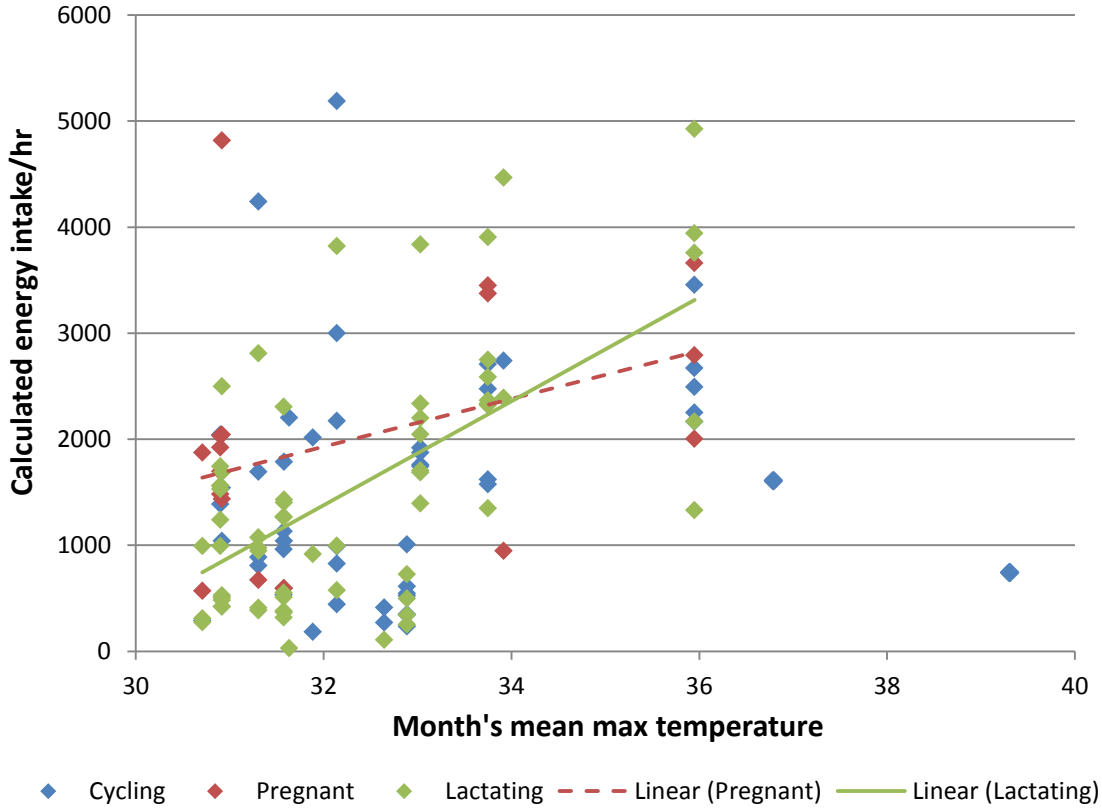

Figure 3.15. Scatter plot showing the effect of reproductive state on the relationship between calculated energy intake rate (kJ/hr) and mean monthly maximum temperature (^{o}C). The lines represent the average linear relationships between the two variables for all pregnant or lactating individuals, as predicted by the GLMM.

However, the lack of relationship between intake rate and maximum temperature for cycling females may simply be due to the fact that the month with the highest maximum temperature (March: 36.8 and 39.3^{o}C for Kwano and Gamgam sites respectively) is represented by just two values, which are both for cycling females, and which appear to disrupt the relationship between maximum temperature and energy intake rate for this group of animals. If the analyses are repeated with these two values removed the interaction effect between maximum temperature and reproductive state becomes marginally non-significant (D=4.87, d.f.=2, p=0.088) and a significant positive relationship for cycling animals is revealed (z=2.26, p=0.024) (figure3.16).

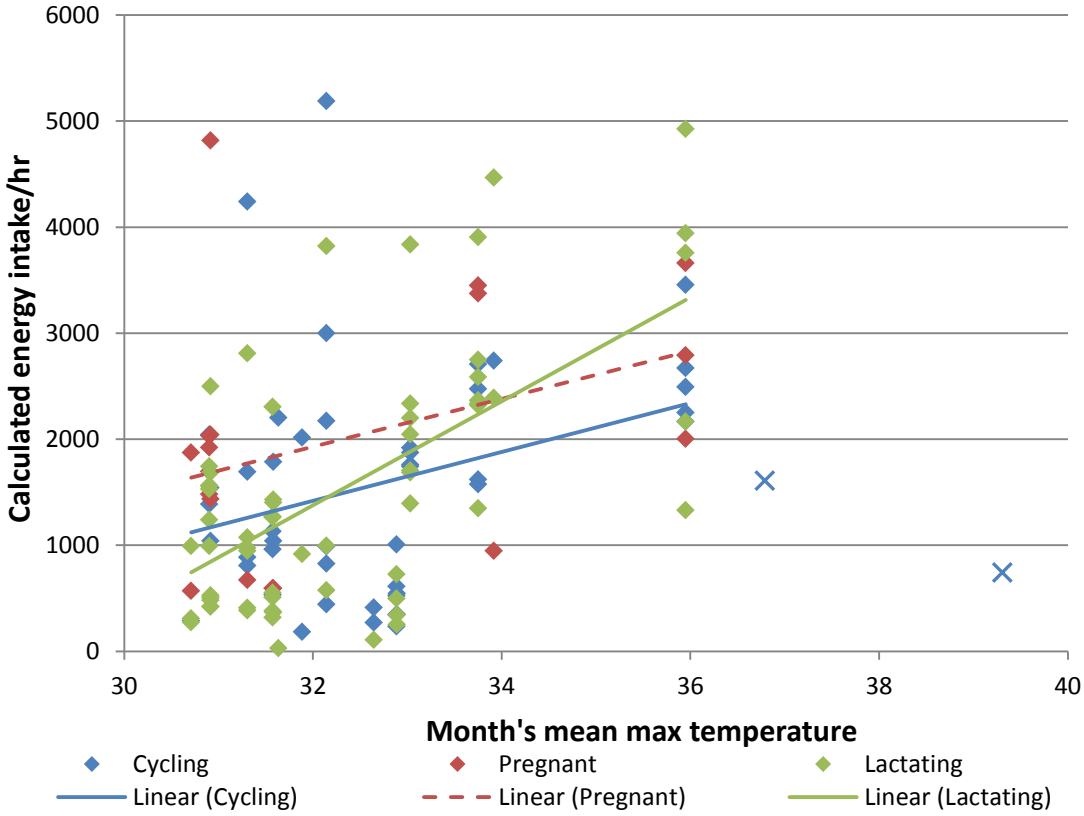

Figure 3.16. Scatter plot showing the effect of reproductive state on the relationship between calculated energy intake rate (kJ/hr) and mean monthly maximum temperature (^{o}C) with 2 data points omitted from analysis, represented by crosses. These represent the only two values collected in March, the hottest month, both from cycling females (focal no. 4, from LMI in Kwano troop and focal no. 97 from BUD in Gamgam troop). The lines represent the average linear relationships between the two variables for all individuals of each reproductive state, as predicted by the GLMM. The line for cycling animals (blue line) omits the two March results in order to demonstrate how, without these values, the relationship between temperature and intake rate for cycling animals is significant and similar to that for pregnant and lactating animals

There was no significant effect of reproductive state on any of the other energy intake rate relationships (appendix 6a, table A6.ii).

Interaction between categorical variables

No significant interaction effect on energy intake rate was found between any combination of troop, season, rank and reproductive success (appendix 6a, table A6.ii). However, the addition of the interaction between rank and reproductive state to the 2-level energy intake rate model containing these variables without their interaction effect

resulted in a marginally non-significant increase in the model's explanatory power (D=8.27, d.f.=4, p=0.082).

High ranking pregnant animals exhibited a higher energy intake rate than high ranking cycling (z=2.08, p=0.038) and lactating (z=3.38, p=0.001) animals but no effect of reproductive state was present for middle or low ranking animals. Within pregnant animals, high ranking animals had higher energy intake rates than low ranking animals (z=2.12, p=0.034) whereas within lactating animals both high (z=2.50, p=0.012) and middle (z=2.06, p=0.04) ranking animals had lower energy intake rates than low ranking animals (figure 3.17, full results in appendix 6a, table A6.iii)

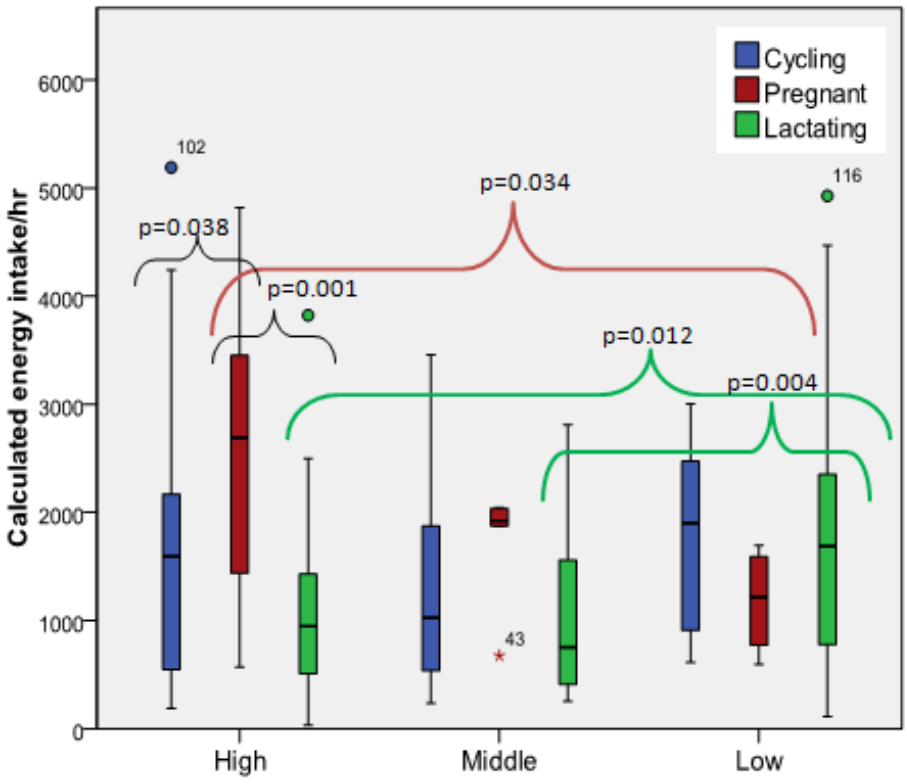

Figure 3.17 Box-plot showing variation in calculated energy intake rate (kJ/hr) between focal animals of different ranks and in different reproductive states.

Crop-raiding behaviour

The calculated energy intake rates of Gamgam troop members did not differ significantly between days when crop food items were observed being eaten (n=11) compared to days when they were not (n=51) (independent t-test: t=-1.33,d.f.=60, p=0.188).

3.2.2.3. Determinants of calculated energy expenditure rate

Weather and fruit index variables

The relationship between calculated energy expenditure rate and the weather and fruit index variables was assessed in the same way as energy intake rate using GLMMs. The addition of daily and monthly maximum temperature and daily and 30-day rainfall (figure 3.18, table 3.12) significantly improved the explanatory power of the energy expenditure rate 2-factor null model. There was a significant positive relationship between energy expenditure rate and maximum temperature (daily and mean monthly) and a significant negative relationship between energy expenditure rate and rainfall (daily and 30-day) (table 3.12, coefficients only included if the model was significantly better than the 2-factor null model).

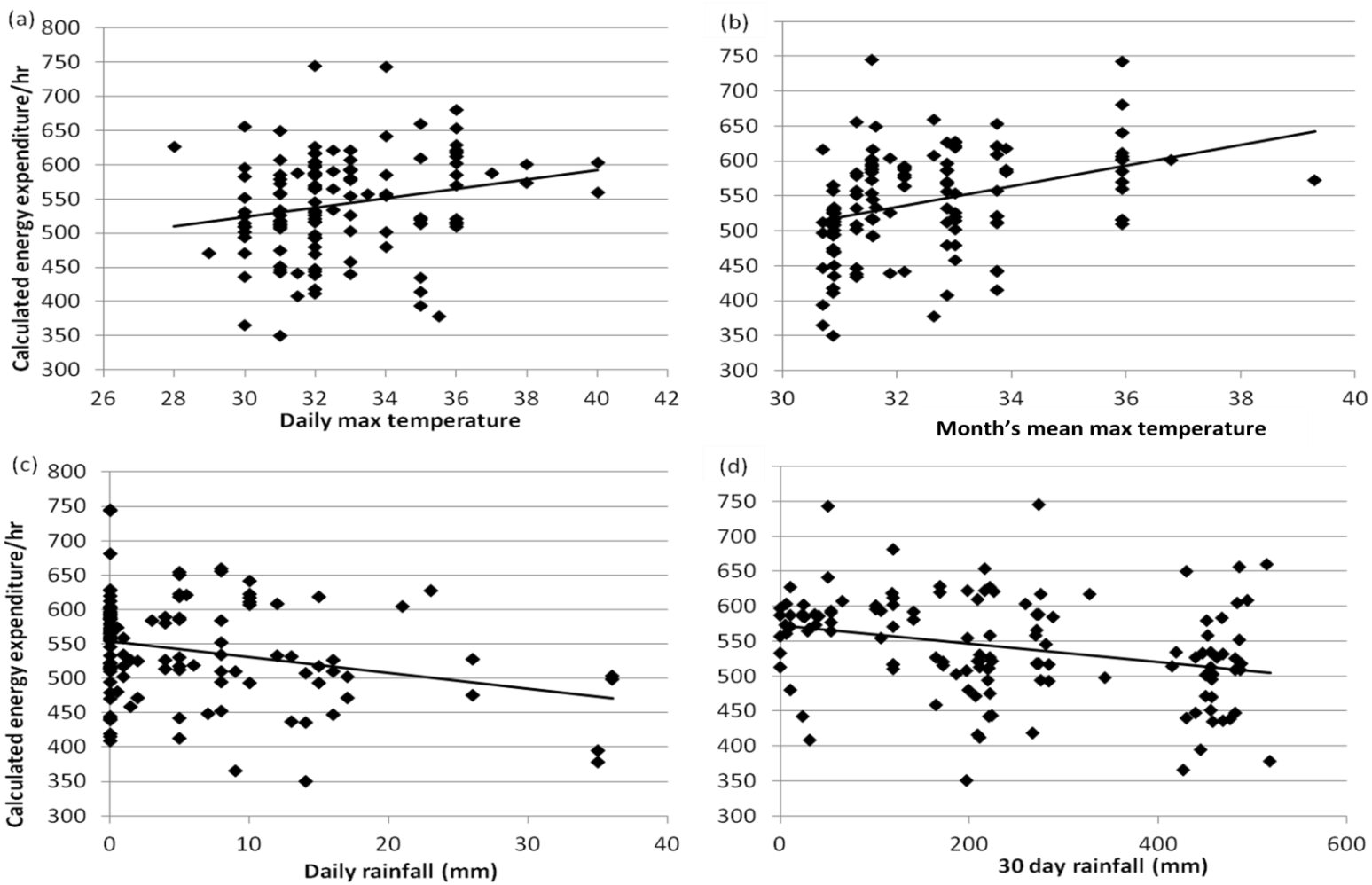

Figure 3.18. Scatter plots showing the relationship between calculated energy expenditure rate (kJ/hr) and measures of maximum temperature (^{o}C) and rainfall. The lines represent the average linear relationships between the two variables for all individuals, as predicted by the GLMM.

Table 3.12. Results of likelihood ratio tests and z tests from GLMMs built to test whether calculated energy expenditure is related to the weather and fruit index variables. Each row represents a separate model.

Explanatory factors	**D[1]**	**p**	**Coefficient**	**s.e.**	**z**	**p**
Daily min temp	0	1				
Daily max temp	5.76	**0.016**	6.885	2.775	2.48	**0.013**
Daily rainfall	9.12	**0.003**	-2.327	0.746	-3.12	**0.002**
Mean monthly min temp	0.01	0.920				
Mean monthly max temp	16.73	**<0.001**	14.852	3.487	4.26	**<0.001**
30-day rainfall	12.39	**<0.001**	-0.133	0.036	-3.69	**<0.001**
Total-fruit index	0.25	0.617				
Tree-fruit index	1.45	0.228				
Vine-fruit index	0.50	0.479				

- D statistic relates to comparison with 2-factor null model
- d.f.=1 in all cases

Season

The addition of season to the 2-level energy expenditure rate null model, as a categorical, fixed variable, resulted in a significant increase in the model's explanatory power (D=12.59, d.f.=1, p<0.001). During the dry season animals experienced significantly higher energy expenditure rates than during the wet season (z=3.64, p<0.001, figure 3.19).

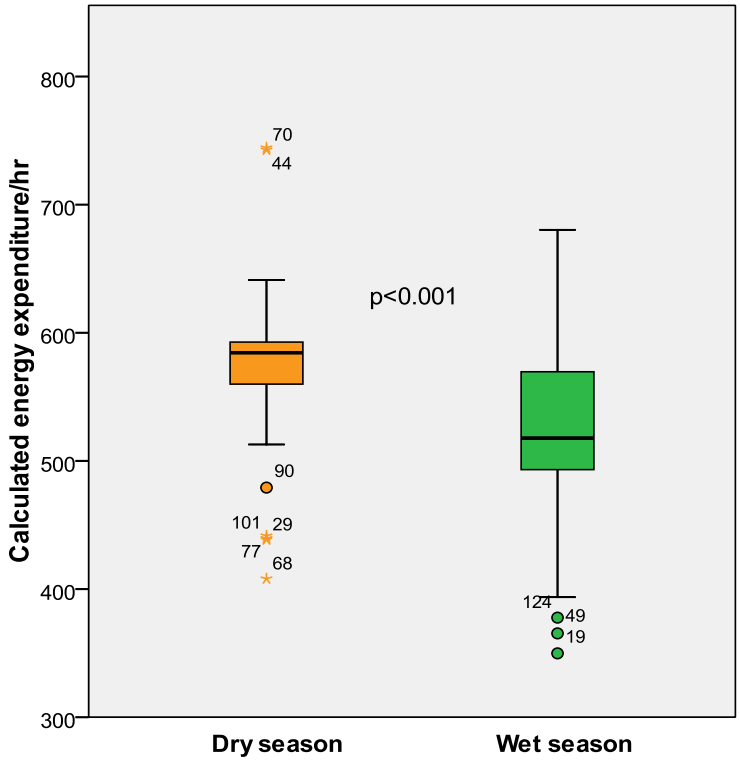

Figure 3.19. Box-plot showing variation in calculated energy expenditure rates (kJ/hr) of focal animals during the dry and wet seasons

The addition of the season interaction effect significantly improved the fit of the energy expenditure rate model containing mean monthly minimum temperature (D=5.14, d.f.=1, p=0.042), revealing a significant positive relationship between expenditure rate and minimum temperature during the wet season (z=2.96, p=0.003) but no relationship during the dry season (z=1.42, p=0.151) (figure 3.20).

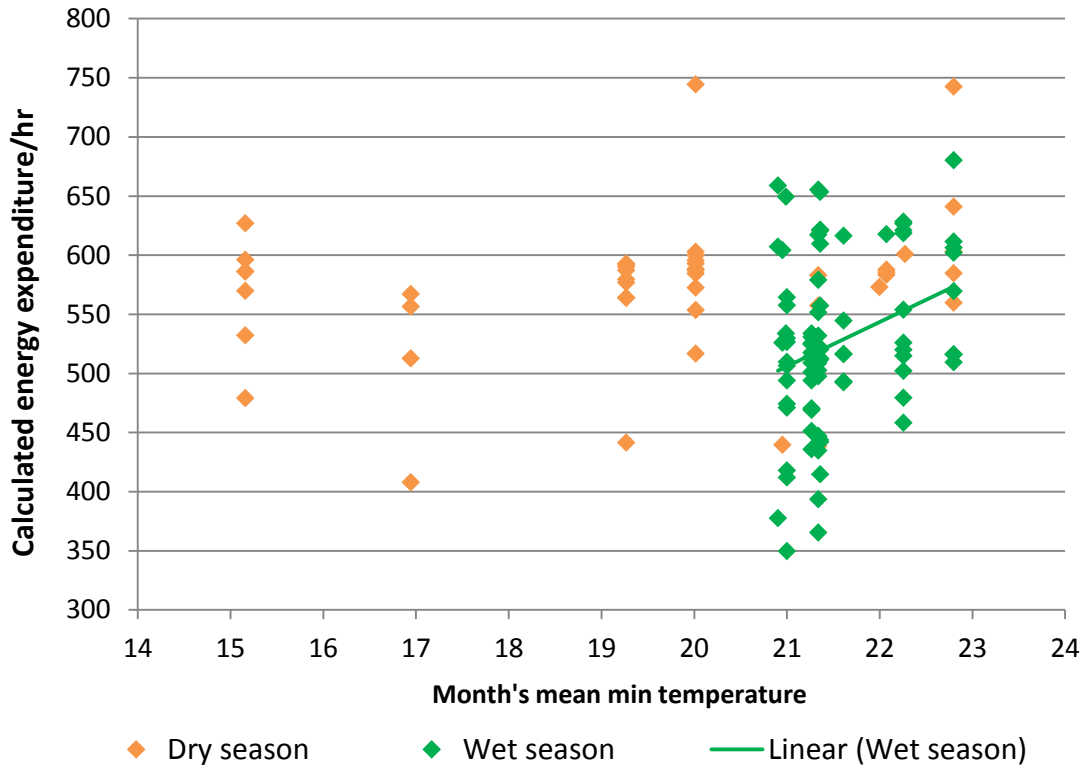

Figure 3.20. Scatter plot showing the effect of season on the relationship between calculated energy expenditure rate (kJ/hr) and mean monthly minimum temperature (^{o}C). The line represents the average linear relationship between the two variables for all individuals during the wet season, as predicted by the GLMM.

There were no significant interaction effects between season and any other of the other weather or fruit index measures (appendix 6a, table A6.iv). However, a z test did reveal a previously unrecognised relationship between energy expenditure rate and vine-fruit index, with a significant positive relationship during the wet season (z=2.76, p=0.006) and a marginally non-significant positive relationship during the dry season (z=1.88, p=0.060). The slope of the relationship was similar in both seasons, which explains why the season-fruit index interaction was not significant (D=0.49, d.f.=1, p=0.483; figure 3.21). A similar effect was found for the closely correlated (Spearman's rank correlation: r_s=0.898, p<0.001) total-fruit index, with marginally non-significant positive relationships between the total-fruit index and energy expenditure rate during the dry (z=1.74, p=0.082) and wet (z=1.77, p=0.078) seasons. The relationship between fruit index and energy expenditure rate was not detected when the data from both

seasons were examined together apparently due to the substantial difference between energy expenditure rates in the two seasons.

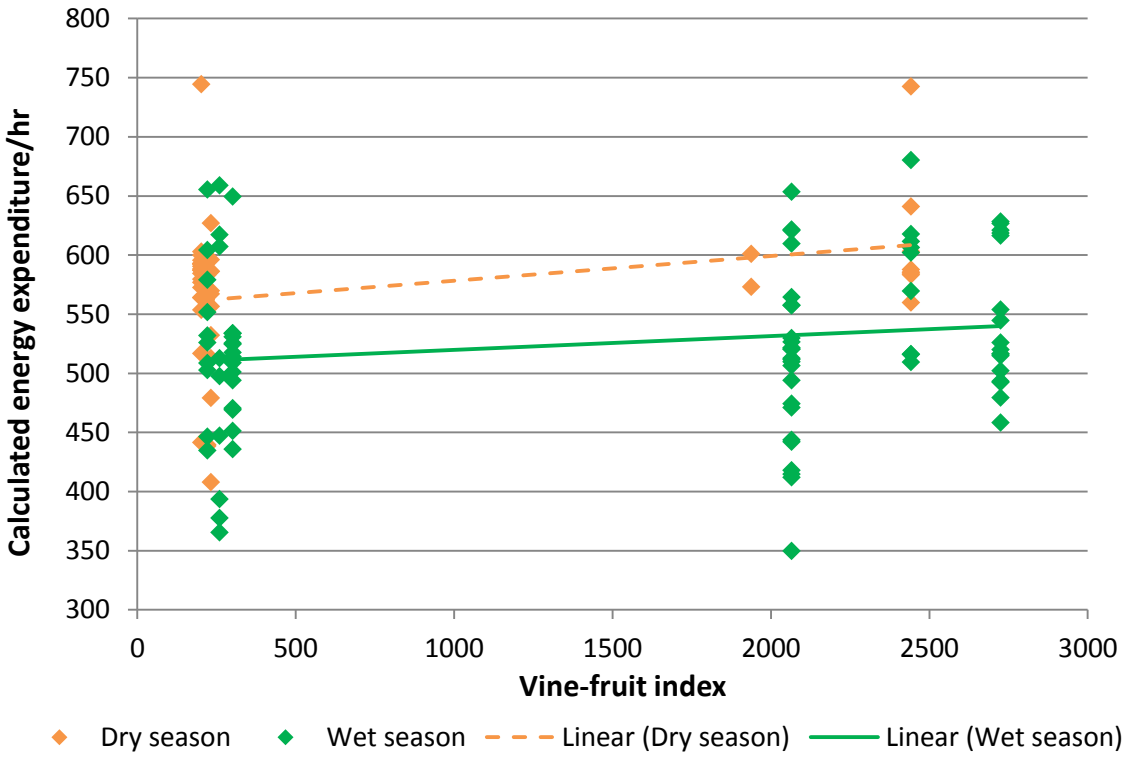

Figure 3.21. Scatter plot showing the effect of season on the relationship between calculated energy expenditure rate (kJ/hr) and vine-fruit index. The lines represent the average linear relationships between the two variables for all individuals during each season.

Troop

The addition of troop to the energy expenditure rate 2-factor null model, as a categorical, fixed variable, resulted in a significant increase in the model's explanatory power (D=8.54, d.f.=1, p=0.003), with Gamgam animals exhibiting significantly higher energy expenditure rates than Kwano animals (z=3.02, p=0.003; figure 3.22).

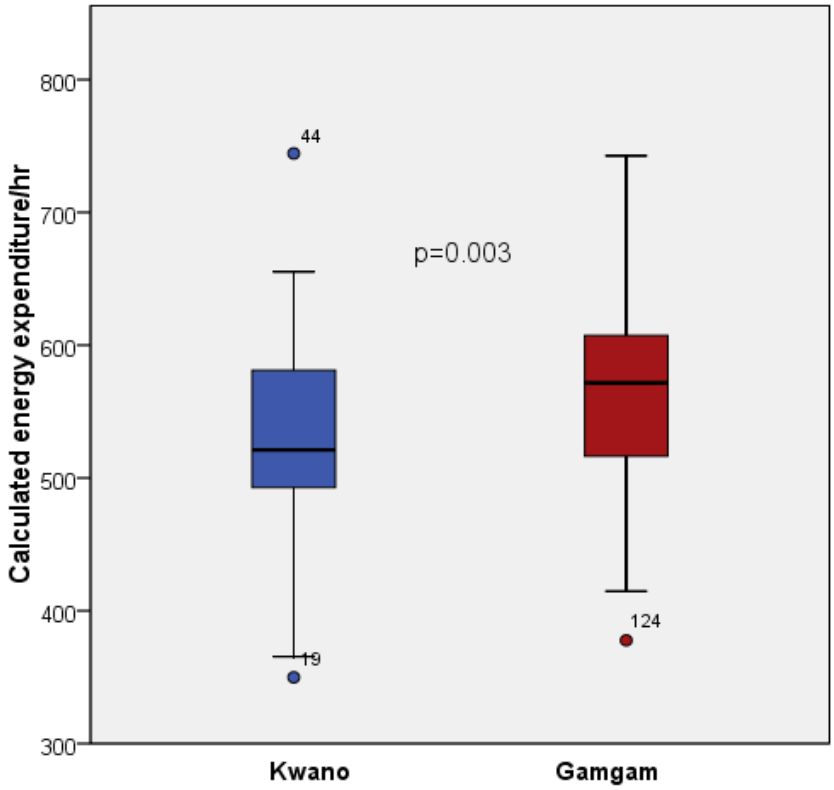

Figure 3.22. Box-plot showing the calculated energy expenditure rates (kJ/hr) of focal animals belonging to different troops.

The addition of the troop interaction effect to the energy expenditure rate model containing mean monthly maximum temperature improved its explanatory power but the effect was marginally non-significant (D=3.22, d.f.=1, p=0.073). There was a significant positive relationship between the two factors for Kwano troop (z=3.19, p=0.001) but not for Gamgam troop (z=1.44, p=0.151) (figure 3.23). The explanatory power of the other models was not significantly affected by the addition of the troop interaction effect (appendix 6a, table A6.iv).

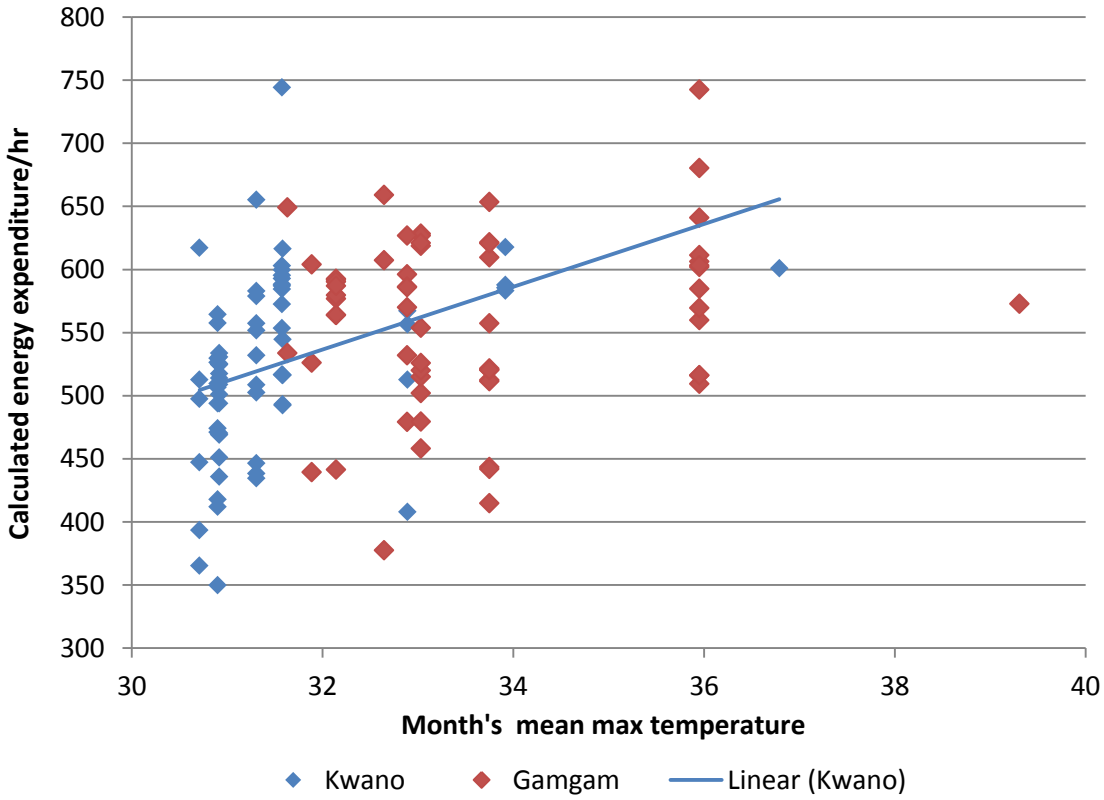

Figure 3.23. Scatter plot showing the effect of troop on the relationship between calculated energy expenditure rate (kJ/hr) and mean monthly maximum temperature (^{o}C). The line represents the average linear relationship between the two variables for all individuals in Kwano troop, as predicted by the GLMM.

Rank

The addition of rank to the energy expenditure rate 2-factor null model did not significantly affect the model's explanatory power, with no significant difference between the energy expenditure rate of high, middle and low ranking animals (D=0.44, p=0.509). The addition of rank and its interaction effect to the energy expenditure rate models containing the weather and fruit index variables had no significant effect on their explanatory power (appendix 6a, table A6.iv).

Reproductive state

The addition of reproductive state to the 2-level energy expenditure rate null model significantly increased the model's explanatory power (D=7.36, p=0.025), with pregnant and lactating animals exhibiting significantly lower energy expenditure rates

than cycling animals (pregnant: z=2.47, p=0.014; lactating: z=2.13, p=0.033) (figure 3.24).

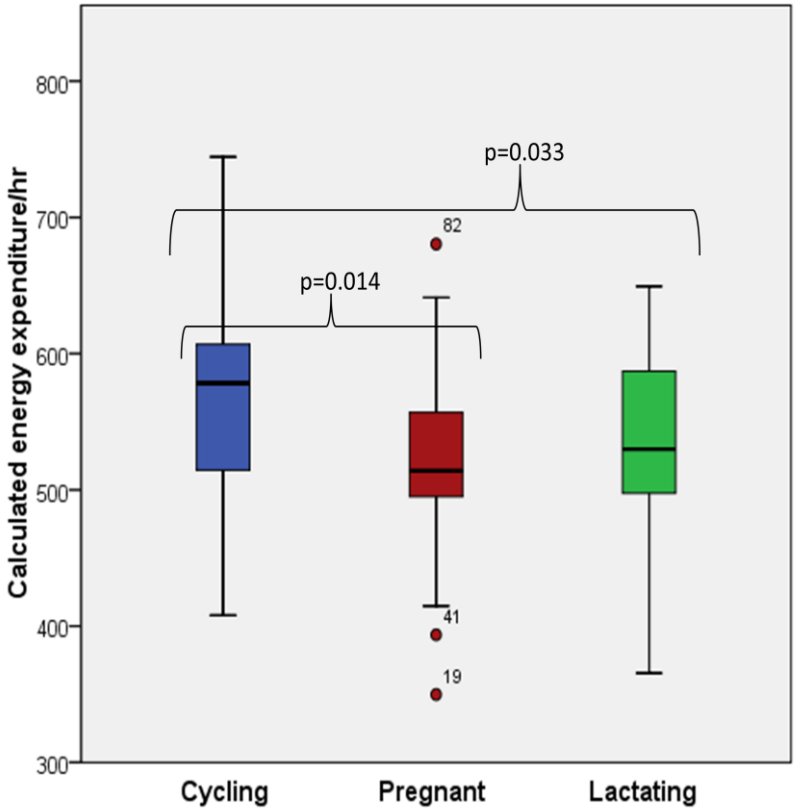

Figure 3.24. Box-plot showing the variation in calculated energy expenditure rate (kJ/hr) between focal animals in different reproductive states.

The addition of the reproductive state interaction effect significantly improved the explanatory power of the energy expenditure rate model containing mean monthly minimum temperature (D=7.09, d.f.=2, p=0.029). A significant and positive relationship was found between energy expenditure rate and minimum temperature for pregnant animals (z=2.59, p=0.010) but not for cycling (z=0.63, p=0.532) and lactating (z=0.74, p=0.462) animals (figure 3.25).

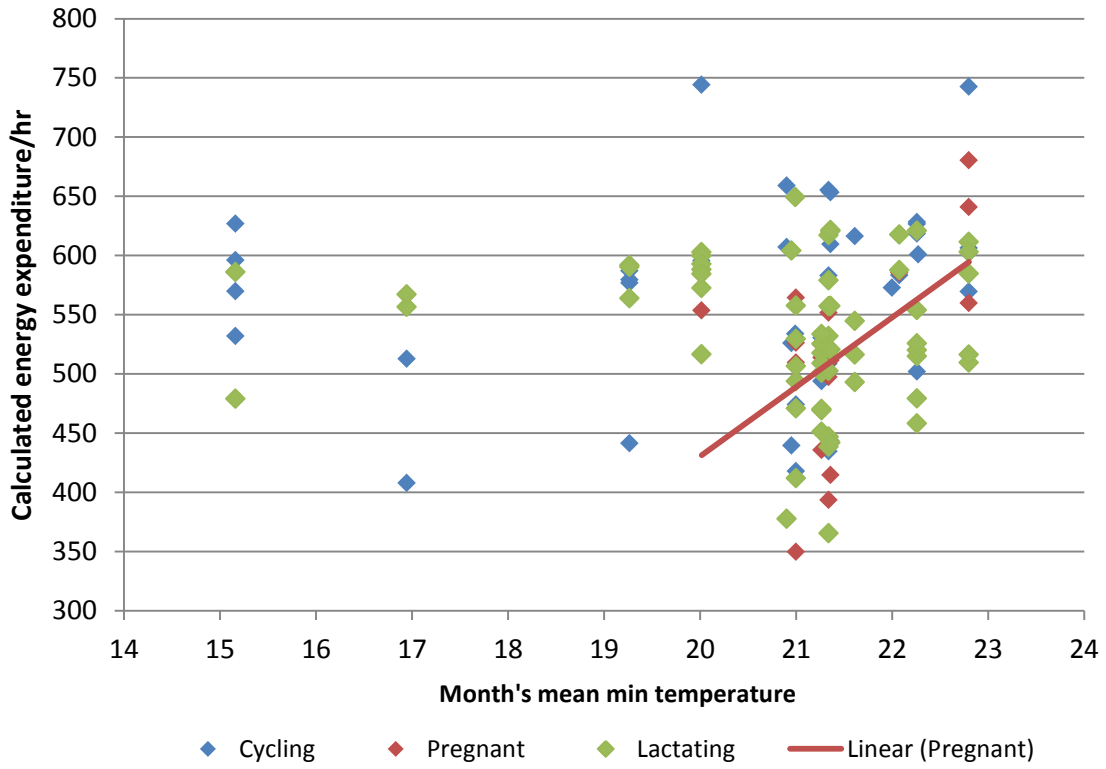

Figure 3.25. Scatter plot showing the effect of reproductive state on the relationship between calculated energy expenditure rate (kJ/hr) and mean monthly minimum temperature (^{o}C). The line represents the average linear relationship between the two variables for all pregnant individuals, as predicted by the GLMM.

Interaction between categorical variables

A marginally non-significant interaction effect was found between troop and season (D=3.46, d.f.=2, p=0.063) and a significant interaction effect was found between rank and reproductive state (D=11.81, d.f.=4, p=0.019). No other combinations of the categorical variables produced significant interaction effects (table 3.13)

Table 3.13. Results of likelihood ratio tests from calculated energy expenditure GLMMs built to test whether any of the categorical variables (troop, season, rank and reproductive state) interact significantly. Each row represents a different model

Explanatory factors	**D**	**d.f.**	**p**
Troop + Season	3.46	1	***0.063***
Troop + Rank	2.39	2	0.302
Troop + Reproductive State	0.64	2	0.728
Season + Rank	3.81	2	0.149
Season + Reproductive State	2.74	2	0.254
Rank + Reproductive State	11.81	4	**0.019**
Troop + Season + Rank + Reproductive state	26.75	29	0.58519

- D statistic relates to comparisons with corresponding 2-factor model without the interaction effect
- Where a model contains two or more explanatory variables the interaction effect is also included

Gamgam troop exhibited higher energy expenditure rates than Kwano troop only during the wet season (wet: z=3.54, p<0.001; dry: z=0.27, p=0.787) and dry season expenditure rates were greater than wet season rates only for Kwano troop (Kwano: z=3.93, p<0.001; Gamgam: z=1.24, p=0.214) (figure 3.26).

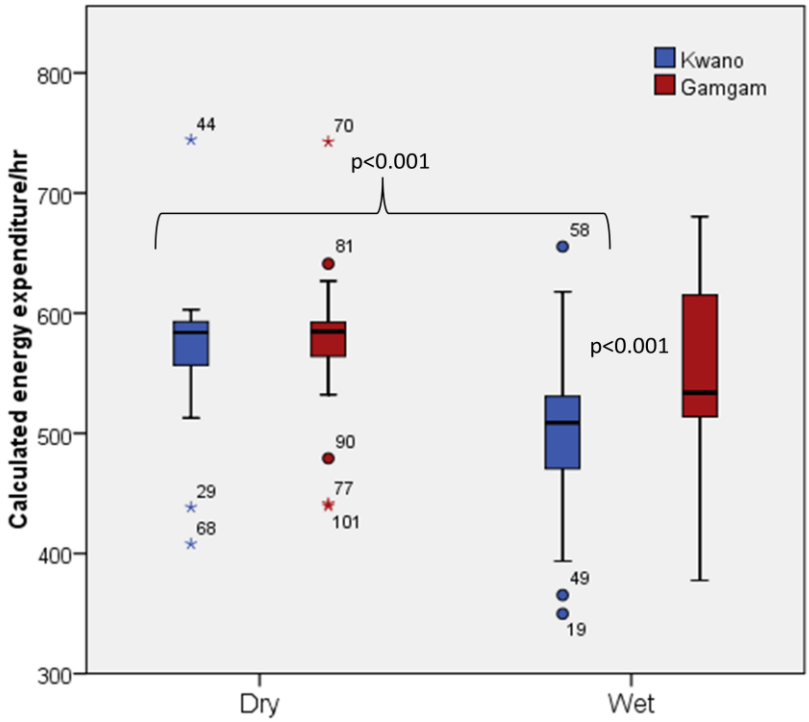

Figure 3.26. Box-plot showing variation in calculated energy expenditure rates (kJ/hr) between focal animals from different troops during different seasons.

Middle and low ranking animals exhibited significantly lower energy expenditure rates when cycling than during pregnancy (middle: z=2.83, p=0.005; low: z=2.28, p=0.023) and, for middle ranking animals only, than during lactation (z=3.20, p=0.001). When cycling, middle ranking animals exhibited significantly higher expenditure rates than high ranking animals (z=2.55, p=0.011) but when lactating they exhibited a trend towards lower rates (z=1.67, p=0.095) (figure 3.27).

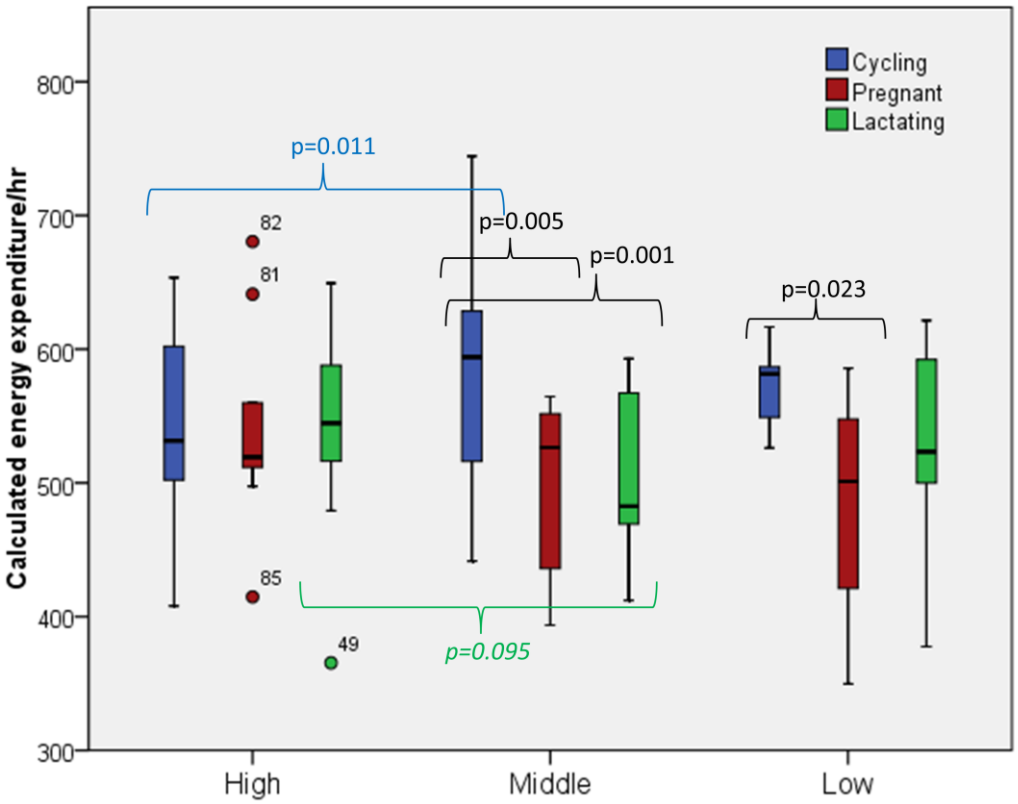

Figure 3.27. Box-plot showing variation in calculated energy expenditure rate (kJ/hr) between focal animas of different ranks and in different reproductive states.

Crop-raiding behaviour

The calculated energy expenditure rates of Gamgam troop members were significantly higher on days when crop food items were observed to be eaten (n=11) compared to days when they were not (n=51) (independent t-test: t=-2.35, d.f.=60, p=0.022, figure 3.28).

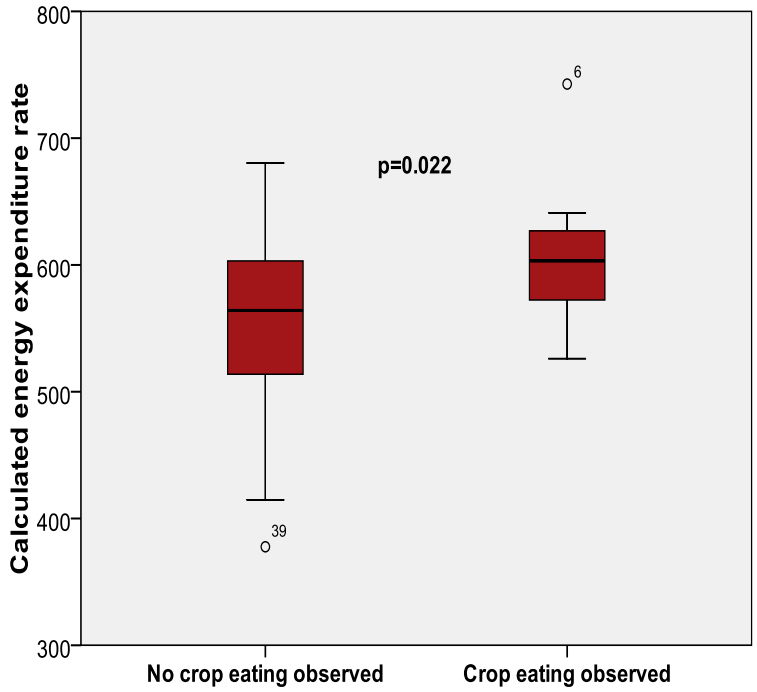

Figure 3.28. Box-plot showing the calculated energy expenditure rates of Gamgam troop focal animals on days when crops were and were not observed being eaten by the baboons.

3.3. Discussion

3.3.1. Activity budgets

3.3.1.1. Effect of troop, season, rank and reproductive success on activity budgets

There was a significant difference between the activity budgets of the two troops. In both the wet and dry seasons, members of Kwano troop spent less time resting and more time feeding than members of Gamgam troop, which is as predicted, but the predicted difference between the proportion of time devoted to travelling and in social behaviours was not found. As discussed in section 3.2.1, a decrease in travel time as a result of food-enhancement is not always found and is not necessarily expected since factors other than food availability, such as water and sleeping site availability and travel to food-enhancement sites, can also affect travel time. The farms which the Gamgam troop raided were located quite a distance from where they tended to forage for wild-food and around 500m from their usual sleeping sites (N. Alberts pers. comm.), so it is not

surprising that travel time was not lower for Gamgam troop. Although the predicted increase in social time was not seen, the fact that Gamgam troop spent as much time in social behaviours as Kwano troop, whilst having fewer members, does suggest that food-enhancement may allow for relatively more social time (Dunbar, 1992).

Season had a significant effect on activity budgets, which was consistent for both troops. During the dry season, more time was devoted to feeding and less time to resting than during the wet season. This is as predicted and consistent with the idea that during periods of lower food availability, i.e. the dry season, more time is spent feeding, since food is less abundant and of lower quality, and therefore less time is available for resting. Travelling time also increased in the dry season, which is as predicted and consistent with the idea that more time must be spent searching for food during the less productive, dry season. The proportion of time spent in social behaviours was higher during the wet season than during the dry season, which is consistent with the idea that more time is available during the more productive, wet season for social behaviours.

There was little effect of rank on the activity budgets, although high ranking Kwano troop animals did spend marginally non-significantly less time travelling than low and middle ranking animals. This is consistent with the rank predictions and a study of vervet monkeys, which found that subordinate females spent more time moving than dominant females but did not differ in the amount of time spent feeding (Isbell and Young, 1993). The lack of effect for Gamgam troop may be due to crop-raiding behaviour weakening the degree of feeding competition, and therefore the costs of low rank, by increasing overall food availability. The two troops also differed in the effect of rank on social behaviours. Within Gamgam troop the single middle ranking animal (BUD) spent significantly more time in social behaviours than the two high ranking

animals. Due to small number of animals in each category this result cannot be used to draw general inferences about the effect of rank on social behaviour. For Kwano troop, middle ranking animals spent less time in social behaviours than high or low ranking animals, although both differences were marginally non-significant. This slightly odd result may reflect the wide variety of behaviours which are contained within the category 'social behaviours'. For example, high ranking females may spend more time being groomed and playing with other females' infants whereas low ranking females may spend more time grooming other animals and begin involved in conflicts.

No effect of reproductive state on activity budgets was found for either troop. This is not as predicted since pregnant and lactating animals were expected either to spend more time feeding or less time travelling (and more time resting) in order to compensate for the increased reproductive costs relative to cycling females. Two factors may explain this lack of difference. First, food availability in the Gashaka baboon's forested habitat is likely to be relatively high, especially in comparison to the savannah habitats experienced by many, better studied, baboon troops (Higham *et al.*, 2009a), which may mean that pregnant and lactating animals do not need to adjust their activity budgets so much in order to obtain sufficient food. Second, pregnant and lactating females may be spending the same amount of time feeding as cycling animals but may be concentrating their time on better quality food and therefore achieving a greater energetic intake in the same period of time.

3.3.1.2. Comparison with 2001/2 activity budget data

The 2009 results, in general, conform to the predictions that Gamgam troop has access to better resources than Kwano troop (and hence spends less time feeding and more time resting) and that food sources are more abundant/ better quality in the wet season

compared to the dry season (and hence less time spent foraging and travelling and more time spent resting and in social behaviours). Warren's (2003) analysis of the Gashaka baboons' activity budgets in 2001/2 found a similar effect of food-enhancement and season on feeding and resting time but she also found that Gamgam troop spent significantly less time travelling and significantly more time in social behaviours than Kwano troop. Another difference between the two studies was that substantially more time was spent feeding and less time resting in the 2009 dataset compared to the 2001/2 dataset.

These differences are likely to be due to a combination of factors. First, the months in which data were collected in the 09 and 01/02 studies do not completely overlap, which could affect both overall activity budgets and the effect of season. Warren collected activity budget data from May-August 2001 and from November 2001-April 2002; no data were collected during September and October. Data collection from the present study ran March-June and August-December 2009; no data were collected during January, February or July. Second, the behavioural sample techniques used in the two studies were different which may have some impact on the activity budgets created. Warren used scan samples whereas in the present study continuous sampling was used. Although both methods can be used to accurately determine the percentage of time animals spend in different activities (Altmann, 1974) continuous focal sampling is recommended over scan sampling for recording detailed behaviours and scan sampling can underestimate rare events (National Research Council, 2003). Third, Warren sampled all members of the troop including adult males and juveniles whereas in the present study only adult females were sampled. This is very likely to have an effect on the overall activity budgets due to the variation in nutritional requirements and activity budgets of animals of different age and sex (section 1.12). For example, in several studies adult males have been found to spend less time feeding than adult females (Alberts *et al.,* 1996; Nakagawa, 2000), which may explain the lower proportion of time spent feeding in the 2001/2 study relative to the 2009 study. On the other hand, juvenile primates

may spend more time feeding than adults so their inclusion in the analysis, in contrast to what was found, would be expected to increase overall feeding time (Rothman *et al.*, 2008). Fourth, the group size of both the troops has increased since Warren's study (modal group size from 2001/2-2009: Gamgam 14-20; Kwano 28-37), which is likely to affect activity budgets. However, increases in group size are expected to increase feeding time, due to increased feeding competition, rather than to decrease it, as was observed (Stacey, 1986). Another possibility is that these differences are simply due to changes or variation in the weather and habitat between the two study periods.

A comparison of the Gashaka baboon activity budgets with similar data from other baboon populations, following Warren (2003), reveals that, like the 2001/2 data, the 2009 Kwano and Gamgam activity budgets are well within the range of mean values from wild-feeding and food-enhanced baboon populations respectively. The effect of food-enhancement on activity budgets is also very similar to the mean effect from other populations (table 3.7), although the reduction in feeding time due to food-enhancement is somewhat lower, which is in line with the idea that the benefits of crop-raiding are less than other forms of food-enhancement (section 1.2.2. and 3.2.1). When it comes to within study site differences in the activity budgets of wild-feeding and food-enhanced baboons the weak effect of crop-raiding as a form of food-enhancement is even more pronounced, with just an 8% reduction in feeding time at Gashaka compared to 20-43% reduction at other sites (table 3.7). Despite these differences, the general structure of both the wild-feeding and food-enhanced Gashaka troops is similar to those found at other sites (table 3.14 after table 5.13 in Warren, 2003).

Table 3.14. Structure of activity budgets for wild-feeding and food-enhanced baboons at Gashaka, during both the 2001/2 and 2009 studies, and at other study sites (adapted from table 5.13 in Warren 2003).

	Study	**% of time spent in activity state**
Wild-feeding troops	Kwano 2009	Feed > Travel > Rest > Social
	Kwano 2001/2	Feed > Travel > Rest > Social
	Other study sites	Feed > Travel > Rest > Social
Food-enhanced troops	Gamgam 2009	Rest > Feed > Travel > Social
	Gamgam 2001/2	Rest > Feed > Travel > Social
	Other study sites	Rest > Feed > Travel > Social

3.3.2. Calculated energy measures

3.3.2.1. Overview of calculated energy balance data

Over three quarters of focal days resulted in positive energy balance and the mean energy balances for both troops were highly positive. However, energy debt (negative energy balance) still occurred on individual focal days in both troops and in both seasons. These results are similar to those found by N'guessan *et al.* (2009) in a study of chimpanzee energy balance where animals experienced negative energy balance on between 9 and 56% of days depending on the season and, for some seasons, mean energy balances equivalent to almost 30,000 kJ/day were found, which is comparable in magnitude the some of the highest energy balance scores found in the current study (e.g. 10% of scores were >15,000kJ).

The fact that the animals in this study and the one by N'guessan *et al.* (2009) often demonstrate such high energy balances, while not appearing to be overweight, may indicate that our techniques for determining energy balance are either over-estimating energy intake or under-estimating energy expenditure or both. There are two major areas in this study which are likely to result in an under-estimation of energy expenditure. Firstly, the energetic cost of digestion and nutrient uptake, thermoregulation, body maintenance (including disease resistance) and, perhaps most importantly for adult females, reproduction (National Research Council, 2003; Schmidt-

Nielsen, 1997; Stelzner, 1988) have not in any way been accounted for and secondly the daily travel distance of the baboons is likely to be under-estimated. Daily travel distance was actually a measure of the observer's daily movements rather than that of the baboons and although efforts were made to match the baboons' movements as closely as possible vertical movement in trees was not accounted for and the movement of the baboons on the ground was also undoubtedly more convoluted than that of the observer, a factor which is known to affect the accuracy of travel distance estimates (Isbell *et al.*, 1999).

Despite the problem of over-estimating energy balance, it is still possible to compare energy balance between troops and seasons if we assume that the degree of over-estimation does not differ between troops and seasons. Kwano animals experienced fewer days of major energy surplus than Gamgam, during both seasons, which provides support for the idea that Gamgam's crop-raiding behaviour provides energetic benefits. This result also reflects the activity budget result which showed that Gamgam troops spent more time resting, and therefore conserving energy, than Kwano troop. The fact that Gamgam troop also spent less time feeding than Kwano troop but still spent more days in energy surplus supports the idea that the raided crops are providing a high quality food source. Both troops experienced more energy surplus days and far fewer energy debt days in the wet season compared to the dry season. This is as predicted and supports the idea that during the wet season food is more abundant due to increased primary productivity.

3.3.2.2. Effect of weather and food availability on calculated energy intake rate

It was predicted that energy intake rate would be greater in the wet season than in the dry season, due to the assumption that food availability would be greater in the season

with higher rainfall and therefore greater primary productivity. It was also predicted that energy intake would be positively related to rainfall, temperature and the fruit indices again due to an assumption that these variables would be positively correlated with food availability (3.2.2).

There was a trend for energy intake rates to be greater during the wet than during the dry season, although this effect was not significant ($p=0.095$). This trend is as predicted and reflects the fact that more energy surplus days and fewer energy debit days were found in the wet season relative to the dry season. The fact that the baboons spent less time feeding during the wet season than the dry season yet were still able to obtain a marginally higher energy intake rate provides evidence for the idea that the food being consumed during the wet season is of higher quality or can be consumed at a faster rate than during the wet season. In contrast to this result, and to the predictions, there was no overall relationship between rainfall and energy intake rates and, during the wet season, there was actually a negative relationship between rainfall and intake rates, which is the opposite of what was predicted. However, there was a trend for increased energy intake rates with higher daily rainfall during the dry season.

The fact that the relationship between rainfall and energy intake rate differs between the two seasons is not surprising considering the difference in the magnitude of rainfall. In the dry season daily rainfall varied from 0mm (on 80% of days) to 10mm whereas during the wet season it varied from 0mm (on 31% of days) to >35mm. The fact that during the dry season there is a trend for daily rainfall to correlate positively with energy intake rate may well reflect the positive relationship between rainfall, primary productivity and food availability which may be particularly relevant at the beginning/end of the dry season as rainfall decreases/increases. The reason why this

does not translate to a similar positive relationship between rainfall and energy intake rate in the wet season may reflect the fact that after a certain amount of rainfall further increases are unlikely to result in such large corresponding increases in primary productivity and therefore food availability (Fay *et al.*, 2008; Schuur, 2003). Higham *et al.* (2009) suggested that the year round high productivity present in the Gashaka baboons' forested habitat may weaken the link between rainfall and food availability. I would add the caveat that the relationship between rainfall and food availability, and therefore energy intake, may still be present for the part of the year when rainfall is low but still variable. The fact that the relationship between rainfall and energy intake is actually negative during the wet season (in contrast to the predicted positive relationship) is most likely related to a decrease in the baboons' activity levels during periods of heavy rainfall. The baboons tended to cease travelling or feeding during heavy rain showers and instead sat still, usually until the rain lessened or stopped (pers. obs.). Higham *et al.* (2009) have suggested that during the wet season rainfall actually limits the amount of time available to the baboons for foraging and other activities. The decrease in energy intake rate seen with increasing rainfall during the wet season is most likely due to an effective decrease in the baboons' active period, caused by the rain, affecting all active behaviours including feeding.

Positive relationships, which did not differ by season, were found between energy intake rate and all temperature and fruit index measures. These results were as predicted due to the importance of fruit in the Gashaka baboons' diet and due to the known relationship between primary productivity and temperature. However, the strong positive relationship between energy intake and temperature alongside the weaker relationship with rainfall requires some explanation, since the correlation between rainfall and productivity in tropical forests is far more established than that between

temperature and productivity (Boisvenue and Running, 2006; Toledo *et al.,* 2011). One possibility is that temperature and energy intake co-vary with a third variable, namely the monthly fruit index. At Gashaka the months of highest fruit availability, as measured by the monthly fruit index, coincided with the periods of highest maximum temperature rather than with the periods of highest rainfall (section 2.11) and the correlation between, for example, monthly maximum temperature and energy intake rate is weaker than the relationship of both these variables with total-fruit index (Spearman's rank correlation, n=130 in all cases: fruit index vs. energy intake/hr: r_s=0.567, p<0.001; fruit index vs. mean monthly max temp: r_s=0.432, p<0.001; energy intake/hr vs. monthly max temp: r_s=0.394, p<0.001). Another possibility is that the baboons required more energy during periods with high maximum temperatures in order to deal with the costs of thermoregulation. The baboon's thermal neutral zone falls between 25 and 30^{o}C, above this temperature the rate of respiratory and cutaneous evaporative water rises rapidly in order to avoid hyperthermia (Stelzner 1988). Although, previous studies examining the relationship between baboon behaviour and temperature have reported decreases in activity levels and shade seeking behaviour at high temperatures rather than increases in feeding or energy intake, the forested habitat of Gashaka means that shade is easy to find and that seeking shade and foraging are not mutually exclusive (Stelzner, 1988; Hill, 2006).

No previous studies have examined variation in the energy intake of baboons throughout seasons or in relation to fruit availability or weather variables, though similar studies for great apes are available. Analyses of chimpanzee (N'guessan *et al.,* 2009) and eastern gorilla (Rothman *et al.,* 2008) diet found no significant variation in the energy intake of adult females between seasons, despite differences in rainfall and the quality and availability of foods. In contrast, the energetic intake of adult female and

male Bornean oragutans (*Pongo pygmaeus*) was found to vary significantly between seasons in response to dramatic fluctuations in fruit availability (Knott, 1998). Since the baboons at Gashaka do not experience anything like the large fluctuations in food availability seen in Borneo, it seems probable that, like the chimpanzees and gorillas, they are able to maintain their energy intake throughout the year despite seasonal variation in food availability, which may explain the minor difference between the energy intake rates of the two seasons. However, the fact that energy intake did vary significantly with the fruit indices does suggest that the Gashaka baboons' energy intake is somewhat vulnerable to variation in food availability.

3.3.2.3. Effect of weather and food availability on calculated energy expenditure rate

As predicted, calculated energy expenditure rates were significantly higher during the dry season than during the wet season and were significantly and negatively related to both rainfall measures. These results are consistent with the idea that higher rainfall is associated with greater food availability, or higher food quality, and therefore less effort is required to find sufficient food. However, this idea is weakened by the fact that the highest levels of fruit availability, as measured by total-fruit index, do not coincide with the periods of greatest rainfall and the lack of relationship between total-fruit index and rainfall (Spearman's rank correlation: 30-day rainfall: r_s=-0.056, p=0.530; daily rainfall: r_s=0.127, p=0.149). However, a strong correlation is present between rainfall and tree-fruit index (30-day rainfall: r_s=0.398, p<0.001; daily rainfall: r_s=0.329, p<0.001) and, in addition to this, more than three quarters of the Gashaka baboons' total feeding time is spent on non-fruit items, the availability of which may also be related to rainfall. For these reasons it remains possible that the negative relationship between rainfall and energy expenditure rate is driven by rainfall's influence on food availability.

The negative relationship between rainfall and energy expenditure rate, and the lower energy expenditure rates during the wet season, helps to account for the higher number of energy surplus days and lower number of energy debt days in the wet season compared to the dry season and is also consistent with the fact that more time was spent resting in the wet season than the dry season. It appears that during the wet season the baboons are able to obtain as much or more energy than they do in the dry season (energy intake rate is marginally non-significantly higher during the wet season) but in a shorter time period (feeding time is reduced in the wet season), presumably due to increased superior food quality (i.e. greater energetic density and lower fibre content) (Dunbar, 1992), and the left over time is spent resting. In this way they appear to be adopting an energy minimising strategy which may be related to two factors. First, as discussed above, heavy rainfall is apparently limiting the baboons' active period during the wet season so more time is spent resting since baboons are unable to spend this time in active behaviours i.e. foraging and travelling (Higham *et al.*, 2009a). The greater availability of higher quality food apparently enables the baboons to maintain their energy intake despite the decrease in the amount of foraging time available to them. Second, it may be that during the wet season the baboons are facing other, hidden, energetic costs in particular the cost of resisting and combating disease. Wet tropical rainforest is considered to be the habitat where primates are most at risk from disease (Freeland, 1976) and positive associations between rainfall and parasite load have been found both within and between populations in several primate species (Milton, 1996; Huffman *et al.*, 1997; Watts, 1998; McGrew *et al.*, 1989).

In addition, a positive association between captive primates' lymphocyte and phagocyte concentrations and the annual rainfall in their natural habitat has been demonstrated,

providing further support for the idea that wetter habitats are associated with greater disease risk (Semple *et al.,* 2002). The Gashaka baboons may be particularly sensitive to these higher parasite loads associated with high rainfall since the species most likely originated in the savannahs of southern Africa and the majority of the species still mostly lives in dryer, less forested habitats (Henzi and Barrett, 2005). It might be expected, therefore, that the Gashaka baboons' white blood cell concentration is lower than would be optimal, given the rainfall of their habitat, and hence that the costs associated with disease resistance and the risk of dying will be greater than for a rainforest adapted species. Indeed, the white blood cell concentration of the two captive baboon taxa (yellow and Hamadryas baboons) were among the lowest of the species tested (Semple *et al.,* 2002). Consistent with this, increased disease risk has been suggested as one of the reasons why baboons do not inhabit the wet forests of central Africa (Kingdon, 1997). Also consistent with this idea, higher levels of both infant and adult baboon mortality, apparently due to disease, have been recorded during the wet season than the dry season at Gashaka (9/13 (69%) of infant and adult female deaths between Jan 2002 and Jan 2006: Higham *et al.,* 2009a). Similarly, during 2009 six out of seven disappearances of Kwano troop members occurred during the wet season. All these disappearances were attributed to death rather than e.g. emigration since three of the individuals were infants or juveniles, one was an adult female (KRM) and the three adult males which disappeared exhibited signs of ill health. It may be that the apparently excess energy observed during periods of heavy rain is in fact being used to resist and combat disease.

Energy expenditure was significantly and positively related to both daily and monthly maximum temperature and, during the wet season only, to monthly minimum temperature. These results are not as predicted for two reasons: first, temperature was

expected to be associated with food availability and is strongly associated with monthly total-fruit index so a negative relationship with energy expenditure was expected since greater food availability would mean that the animals would not need to travel so far to satisfy their energy requirements. Second, the risk of hyperthermia associated with temperature >30^{o}C has, in other baboon studies, been associated with decreased activity levels rather than increased activity levels (Stelzner, 1988; Hill, 2006). Although this relationship may be due to a confounding, third variable, a suitable candidate cannot be identified at this point. For example, 30-day rainfall correlates negatively with both mean monthly maximum temperature and energy expenditure rate (Spearman's rank correlation: 30-day rainfall vs. energy expenditure r_s=-0.295, $p<0.001$; 30-day rainfall vs. monthly max temp r_s=-0.597, $p<0.001$) but the addition of 30-day rainfall to the energy expenditure rate model containing maximum temperature does not significantly improve its fit (D=2.20, d.f.=1, p=0.138) and the relationship between expenditure rate and maximum temperature remained significant (z=2.59, p=0.010).

Although no significant relationship between energy expenditure rate and any of the fruit indices was detected when data from the whole study period were examined together, the addition of season to the model revealed a positive relationship with both vine- and total-fruit index of similar slope for both seasons. This result is in contrast to the predictions since higher food availability was expected to be negatively associated with the amount of energy which needed to be expended in order to find food. However, the relationship between energy expenditure and food availability is not always easy to predict. When faced with low food availability one of two contrasting strategies may be adopted: increase travel time/distance in order to obtain more food or decrease travel time/distance in order to save energy (Dunbar, 1988; Malenky and Wrangham, 1994). The negative relationship between rainfall, as a proxy for food availability, and energy

expenditure suggests that the Gashaka baboons are adopting the former strategy, since they are travelling further during periods of low rainfall, whereas the positive relationships between energy expenditure and both temperature and fruit index, in contrast, seem to suggest that the Gashaka baboons are adopting the latter, energy saving, strategy. It may be that something more complicated is going on. For example, N'guessan *et al.* (2009) found seemingly contradictory results when they examined the relationship between day journey length (DJL) and food availability in chimpanzees. During the most unfavourable season (in terms of food availability and food quality) DJLs were significantly shorter than during the most favourable season, which is consistent with the energy saving strategy. However, within the unfavourable season, there was a significant negative relationship between food availability and DJL, consistent with the idea that animals are travelling further differences to obtain the necessary food (N'guessan *et al.,* 2009). Results from other sites and species are also varied. For example, Hill and Dunbar (2002) found no relationship between the composition of baboon diets and either time spent travelling or day journey length but positive relationships between food availability and day journey length have been demonstrated in chimpanzees (Doran, 1997).

3.3.2.4. Effect of categorical variables on energy intake and expenditure rates

Troop

Energy intake rates were significantly higher amongst the Gamgam troop animals compared to the Kwano animals, which is as predicted. The fact that Gamgam troop animals manage to achieve a higher energy intake rate despite spending less time feeding than Kwano troop supports the idea that their crop-raiding behaviour is providing a large energetic advantage over a diet based purely on wild-foods. This result also suggests that crop foods provide more energy per unit feeding time than wild-foods

and indeed the two most important crop food items (maize cobs and mango fruit) provide Gamgam troop with around six times more energy per minute than the average food item sampled for this study, a difference due both to a greater energy density (c. 1.5 times) and a much greater mass feeding rate (c. 4 times) than the average (table 3.15).

Table 3.15. Comparison of the energetic quality of the two most important crop-food items to average values for all sampled baboon foods.

	Mean of all sampled items	**Maize[1]**	**Mango[2]**
Energy content (kJ/100g)	1000.0	1502.5	1494.4
Mass feeding rate (g/min)	7.15	28.43	27.58
Energy intake rate (kJ/min)	71.49	427.1	412.2

1. *Zea mays* cob, food item no. 55
2. *Mangifera indica* fruit, food item no. 57

In contrast to predictions, Gamgam troop also exhibited significantly higher energy expenditure rates than Kwano troop. This may well be related to the high energetic costs associated with crop-raiding behaviour and, indeed, Gamgam troop did exhibit significantly elevated energy expenditure rates on days when crops were observed being eaten relative to other days. This result suggests that Gamgam's higher energy intake rate comes at a cost, thereby weakening the case for Gamgam's superior energetic status and the energetic benefits from crop-raiding. However, the effect of troop on intake rate was far larger than the effect on expenditure rate: Gamgam troop took in, on average, 44% more energy per hour than Kwano troop yet expended only 7% more energy. The fact that Gamgam troop was able to achieve such an elevated energy intake rate, relative to Kwano troop, whilst expending only a little more energy, is, therefore, still consistent with the idea that crop-raiding behaviour provides an energetic advantage to Gamgam troop. Also consistent with this idea, and the predictions, is that Gamgam troop experienced a higher proportion of energy surplus days than Kwano troop. Although

many studies have demonstrated a beneficial effect of food-enhancement on primate activity budgets behaviour, in terms of reduced feeding/travel time and increased resting time (see references in chapter 1 and section 3.3.1), this is the first to examine the effect of food-enhancement on the energy intake and expenditure of individual animals and the first to provide evidence, in this way, for an energetic advantage of food-enhancement.

The relationships between energy intake rate and the weather and fruit index variables were the same for both troops. This result was not necessarily expected since food-enhancement has been shown to provide a buffer against environmental influences on wild-food availability (Bronikowski and Altmann, 1996). However, the availability of crops is affected by weather variables, unlike the availability of food from other kinds of food-enhancement such as rubbish-raiding, and so weather is still likely to influence food availability for crop-raiding primates such as Gamgam troop.

The relationships between energy expenditure rate and the weather and fruit index variables were also mostly the same for both troops, supporting the idea that despite the effect of food-enhancement Gamgam troop's behaviour and energetic status are still closely linked to environmental variables. However, a positive relationship between energy expenditure rate and monthly minimum temperature was found only for Kwano troop. As discussed above, the positive relationship between energy expenditure rates and temperature was not predicted and is difficult to explain. However, the fact that this relationship is significant for Kwano troop but not Gamgam troop may well reflect the predicted weaker association between environmental factors and energy expenditure for the crop-raiding troop.

Rank

Animals of different social ranks did not differ significantly in terms of either their energy intake or energy expenditure rates, reflecting the lack of difference in their activity budgets. However, interaction effects between rank and reproductive state were present for both energy intake and expenditure rate, including the predicted rank effect on the intake rates of pregnant animals (see 'Interaction between categorical variables' section below for more details). This weak effect of rank was not as predicted since the benefits of high rank have been shown to include better access to high quality feeding sites (Barton, 1993) and foods (Altmann and Alberts, 2005) and higher rates of nutrient acquisition (Barton and Whiten, 1993) in baboons and higher rates of energy intake and net energy gain in other primates (Koenig, 2000; Vogel, 2005). The baboon studies mentioned here all took place in savannah habitats. Perhaps at Gashaka the generally high levels of food availability, due to the high rainfall and forested environment, reduce the level of feeding competition and therefore its negative influence on low ranking animals and perhaps even on the strength of the dominance hierarchy itself (Barton *et al.*, 1996; Garcia *et al.*, 2006).

The relationships between both energy intake and expenditure rate and almost all the weather and fruit index variables were the same for animals of all ranks. The only exception was the relationship between energy intake rate and mean monthly maximum temperature, which was significant and positive, for high and low ranking animals but not for middle ranking animals. This result most likely reflects the fact that energy intake rates were substantially higher amongst Gamgam animals, compared to Kwano animals, alongside the fact that there was only one middle ranking Gamgam animal.

These results suggest that the dominance hierarchy at Gashaka has little influence on individuals' energy intake and expenditure which means that rank related differences in fitness between animals of different ranks, if there are any, could not be driven by energetic variation and instead would relate to other factors such as psychosocial stress (Garcia *et al.,* 2006). In later chapters in this thesis the effect of rank on physiological stress levels, reproductive hormones and reproductive output will be investigated.

An alternative explanation is that this lack of result may be due to measurement bias or lack of detail, for example the energetic expenditure of lower ranking animals could have been underestimated if they spent more time climbing trees and meandered more while travelling (e.g. due to having search harder for food because of exclusion from high quality feeding sites by higher ranking animals (e.g. Barton and Whiten, 1993)) or were more restless than higher ranking animals (e.g. due to disruption by higher ranking animals and generally higher anxiety levels (Higham *et al.,* 2011a)).

Reproductive state

Pregnant animals exhibited lower energy expenditure rates and a trend towards higher energy intake rates relative to cycling animals, which is as predicted, due to the extra energetic demands of pregnancy, and is consistent with other studies of baboons showing increased food consumption (Silk, 1987) and reduced activity levels (Altmann, 1980; Barrett *et al.,* 2006) in pregnant animals relative to cycling animals. Lactating animals also had significantly lower energy expenditure rates than cycling animals, as predicted, but did not exhibit the expected elevation in energy intake rates, instead having similar intake rates to cycling animal and marginally non-significantly lower rates than pregnant animals. Given that the energetic costs of lactation are greater than those of pregnancy (National Research Council, 2003) it is surprising that lactating

baboons do not appear to be adjusting their energy intake and expenditure to the same degree as pregnant animals. It may be that the lactating animals at Gashaka are less able to make up for their extra energetic costs than pregnant animals because the presence of a dependent infant decreases their foraging efficiency (Silk, 1987). The calculation of energy expenditure for this study did not take into account how infant carrying increases the energetic costs of travelling and foraging (Altmann and Samuels, 1992) and therefore the decrease in energy expenditure rates of lactating animals relative to cycling animals measured in this study may be negated by these unmeasured costs. Given these issues, we would expect the Gashaka baboons to exhibit some physiological or physical cost of lactation such as weight loss, as has been observed for lactating chacma baboons (*P. h. ursinus*) in South Africa (Barrett, 2006).

The relationships between both energy intake and expenditure rate and almost all the weather and fruit index variables were the same for animals of all reproductive states. The relationship between energy intake rate and mean monthly maximum temperature did vary significantly between the reproductive states, with a significant relationship for lactating animals, marginally non-significant relationship for pregnant animals and no sign of a relationship for cycling animals. If maximum temperature is related to food availability (as it is to vine-fruit index) this result may reflect the fact that animals with higher energetic demands (i.e. pregnant and lactating animals) take greater advantage of the increases in food availability to increase their energy intake, since they are in greater need. This also means that when food availability is low, pregnant and, to an even greater degree, lactating animals may be less able to obtain energy intake rates equivalent to those of cycling females perhaps due to decreased foraging efficiency. Alternatively, if higher maximum temperatures are associated with increased costs of thermoregulation, perhaps these costs are greater for pregnant and lactating animals due

to greater body mass and physiological demands (pregnant animals) or increased travel costs (infant carrying, lactating females) and hence require greater increases in energy intake for these animals. Another possibility is that the lack of relationship between intake rate and maximum temperature for cycling females is due to un-equal distribution of sample between months, with different maximum temperatures, and reproductive states (figure 3.16).

Interaction between categorical variables

An examination of the interactions between the categorical variables must be approached with caution due to the small number of data points which occur in each category, once more than one categorical variable is considered. For example, there is only one animal in each of the following categories: middle ranking, Gamgam troop; pregnant, Gamgam troop; middle ranking, pregnant. This is the reason why the effect of multiple categorical variables on the relationships between the energy measures and the weather and fruit index variables were not considered at the same time.

For energy intake rate the only interaction effect that approached significance occurred between rank and reproductive state. Amongst pregnant animals, high ranking animals exhibited higher energy intake rates than low ranking animals, which is consistent with predictions about rank and the expectation that higher ranking animals have better access to high quality food resources. However, amongst lactating animals, energy intake rates were significantly higher for low ranking animals compared to those of both high and middle rank, which is the opposite of the rank predictions. This result is difficult to explain but it could be that low ranking animals, perhaps in worse condition, require more energy to offset the costs of lactation, or the result could simply be an artefact of the small sample sizes or inaccuracies/biases in the measures used. This

interaction model also revealed an effect of reproductive state on energy intake rate. The rates for pregnant animals exceeded those of both lactating and cycling animals only within high ranking animals; reproductive state had no effect for either middle or low ranking animals. These results seem to suggest that only high ranking pregnant animals are able to offset the costs of pregnancy by increasing energy intake rates and that lactating animals of all ranks are equally unable to increase their intake rates to offset the extra costs.

Rank and reproductive state also interacted significantly for energy expenditure rate. Amongst cycling animals, high ranked animals exhibited significantly lower energy expenditure than middle ranked animals, as predicted, but amongst lactating animals this pattern was reversed, with higher expenditure rates for high ranked animals. Also in contrast to predictions, low ranked animals in all reproductive states did not differ significantly from either middle or high ranked animals. There was no effect of reproductive state on the expenditure rates of high ranking animals but for the lower ranked animals pregnancy and lactation, as expected, corresponded with lower energy expenditure compared with cycling animals.

When data from all the ranks were considered together, pregnant animals exhibited significantly higher energy intake rates and significantly lower energy expenditure rates than cycling animals, as was predicted. The results of these rank-reproductive state interaction models seem to suggest that high ranking animals offset the extra costs of pregnancy by increasing energy intake rates, while maintaining the same energy expenditure rate, whereas middle and low ranking animals maintain similar intake rates, perhaps because they are unable to increase them, and instead offset the costs of pregnancy by reducing energy expenditure rates. This difference may reflect lower

ranking animals' worse access to resources, poorer body condition or inferior foraging efficiency (see section 1.2.3 for references).

For energy expenditure rate, there was a marginally non-significant interaction between troop and season, with the season effect (dry>wet) only occurring for Kwano troop and the troop effect (Gamgam>Kwano) only occurring during the dry season. The lack of season effect for Gamgam troop supports the idea that this troop's crop-raiding behaviour provides some buffer against environmental effects. This result may also help to explain the unpredicted troop effect, whereby Gamgam, the food-enhanced troop, exhibits higher energy expenditure than Kwano troop but only during the dry season. While Kwano troop substantially reduces expenditure rates during the wet season, perhaps to conserve energy needed to combat higher disease risks, Gamgam troop's expenditure rate remains fairly constant throughout the year, perhaps due to lower disease risks associated with a less wet and forested habitat or due to the benefits of crop-raiding, which means that during the wet season expenditure rates are higher at Gamgam troop than at Kwano.

3.4. Summary of results in relation to original hypotheses

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits

- The observed difference in the activity budgets of Kwano and Gamgam troops reflects those found in other studies of wild-feeding and food-enhanced baboon troops and the idea that crop-raiding provides energetic benefits. However, as predicted, the effects are less pronounced than for other forms of food-enhancement.

- Higher numbers of energy surplus days and increased energy intake rates supports the idea that Gamgam troop's crop-raiding behaviour provides an energetic benefit over a purely wild-food diet.

Hypothesis 2: Within troops, the baboon's condition will vary according to reproductive state and rank

- There was little evidence for an effect of rank on the Gashaka baboons' activity budgets or calculated energy intake and expenditure rates
- Activity budgets differed little between female baboons of different reproductive states. In contrast, pregnant and lactating animals reduced their energy expenditure rates relative to cycling animals and pregnant animals also showed signs of elevated energy intake rates.

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability

- Seasonal differences in the Gashaka baboons' activity budgets are as predicted and reflect the link between seasonal variation in weather, food availability and primate behaviour.
- Evidence is presented which suggests that high levels of rainfall and productivity in the habitat have loosened, but not entirely removed, the link (found in other populations) between rainfall, food availability and energy intake for the Gashaka baboons.
- The importance of factors other than food availability, such as disease risk during heavy rain and thermoregulatory costs during high temperatures, in influencing both energy intake and expenditure are discussed.

Chapter 4

URINARY C-PEPTIDE AS AN INDICATOR OF ENERGETIC STATUS

4.1. Introduction

Despite the importance of energetic status as a key component of individual fitness, its measurement in wild animals can be extremely difficult. Direct measurement of body condition, to obtain BMI or morphometric measurements, tends to be very invasive, as capture and sedation are normally required (e.g. Altmann *et al.* 1993; Cambell and Gerald, 2004). Remote weighing of wild primates to determine body mass is possible but usually involves baiting scales with food, which has the potential to disrupt natural feeding and social behaviours, as well as having a direct effect on the animals' energy intake (Dittus, 1998; Cooper *et al.*, 2004; Pusey *et al.,* 2005). Other less invasive methods offer only crude measures of energetic status. Several researchers have used subjective measures for scoring the body condition or fatness of individual primates based on their visual appearance (e.g. Berman and Schwartz, 1988; Johnson and Kapsalis, 1995) and others have tested for the presence of ketones in urine which, as a product of the breakdown of fat, are produced during severe negative energy balance (e.g. Knott, 1998; Kaur and Huffman, 2004). These two techniques are only able to detect large differences or changes in body condition. A relatively new development in this field is the measurement of C-peptide in urine, which provides a non-invasive method for the quantification of physiological energy balance in wild primates. However, research has so far been limited to a few species and research into the applicability of this technique to a variety of primate species and for use in remote field sites is still required.

4.1.1. C-peptide physiology

Insulin synthesis involves the production of the proinsulin molecule, which consists of an A and B chain linked by a connecting (C) peptide, which serves to align the two chains in order for disulphide bonds to form between them. Proinsulin molecules are packed into secretory granules where proteolytic enzymes cleave the C-peptide from the A and B-chains to form insulin (figure 4.1). The secretory granules, containing equal numbers of insulin and C-peptide molecules, are then secreted into the blood in response to appropriate stimuli (Espinal, 1989; Ido *et al.*, 1997).

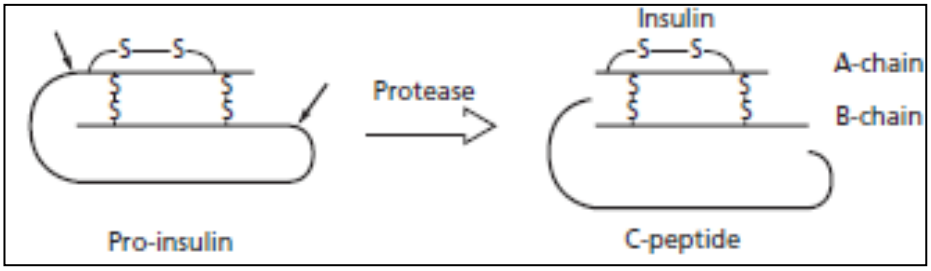

Figure 4.1. Diagram showing the formation of C-peptide during the biosynthesis of insulin (from Holt and Hanley, 2010)

The primary stimulus is the presence of glucose in the blood, from digested carbohydrates, but the presence of amino acids from ingested proteins, can also stimulate insulin secretion (Nuttall and Gannon, 1991). Although ingested fat does not directly stimulate insulin secretion it can affect both glucose blood levels and the response of insulin to carbohydrate ingestion, increasing the strength of the insulin response to blood glucose (Collier and O'Dea, 1983). Beyond its role in insulin synthesis C-peptide is generally considered to possess little biological activity, although recent studies have revealed some physiological effects (Wahren *et al.*, 2007). However, the measurement of C-peptides in blood or urine has proven to be a very useful indicator of insulin production in human clinical studies (e.g. Bonser and Garcia-Webb, 1984; Miura *et al*., 1996; Suzuki *et al.* 2004; Goetz *et al.* 2002), due to the fact that the

two molecules are produced in equimolar amounts and because C-peptide breaks down far more slowly than insulin (Horwitz *et al.,* 1975).

4.1.2. Urinary C-peptides as an indicator of energetic status

Insulin plays a key role in the regulation of energy balance by controlling the use of glucose by tissues and the storage of glucose as glycogen and fat (Holt and Hanley, 2010). Insulin, and therefore C-peptide, can act as a sensitive indicator of an individual's energetic status since it tracks variation in the balance between energy use and storage (Polonsky *et al.,* 1988; Doucet *et al.,* 2000; Yoshida *et al.,* 2006). The biological inertness of C-peptide in the blood and the fact that a consistent fraction (c.5%) is excreted, un-metabolised, in urine means that the measurement of urinary C-peptides (UCPs) offers a non-invasive method for quantifying an individual's energetic status (Horwitz *et al.,* 1975; Meistas *et al.,* 1982; Sherry and Ellison, 2007).

In humans, UCP levels have been shown to correlate positively with body mass index (Polonsky *et al.,* 1988) and, in a study of recovering anorexic patients, with calorific intake (Yoshida *et al.,* 2006). UCPs have also been used in anthropological field studies, where a gradual increase in UCPs, from initially low levels at the start of lactation, until resumption of full ovarian function, has been identified as evidence that maternal energetic status is the main determinant of lactational amenorrhea (Ellison and Valeggia, 2003; Valeggia and Ellsion, 2009). The results of these studies provide support for the use of UCPs as an indicator of both inter- and intra-individual variation in energetic status (Deschner *et al.,* 2008).

Far fewer studies have investigated the use of UCPs as an indicator of energetic status in non-human primates (Sherry and Ellison, 2007). In laboratory studies of rhesus

macaques, UCPs have been shown to correlate with insulin production (Kemnitz *et al.,* 1994) and with energetic status (Wolden-Hanson *et al.,* 1993). For example, obese macaques exhibited higher UCP levels than non-obese macaques and food deprivation resulted in decreases in UCP levels (Wolden-Hanson *et al.,* 1993). More recently UCPs have been successfully used as an indicator of individual energetic status in free-ranging and captive great apes and non-hominid primates where UCP levels have been shown to co-vary with both body condition and energy intake (table 4.1).

Urinary C-peptide levels co-varied with body mass in both captive bonobos and captive macaques during food restriction and re-feeding experiments (Deschner *et al.,* 2008; Girard-Buttoz *et al.,* 2011) and were positively correlated with BMI and skinfold fatness in captive and free-ranging macaques (Girard-Buttoz *et al.,* 2011). Positive correlations between UCP level and energy availability (i.e. food intake, availability, abundance or quality) have been found in wild Bornean orangutans (Sherry and Ellison, 2007; Emery Thompson and Knott, 2008), chimpanzees (Sherry and Ellison, 2007; Emery Thompson *et al.,* 2009), and bonobos (Georgiev *et al.,* 2011), in captive rhesus and long-tailed macaques (Girard-Buttoz *et al.,* 2011) and in wild black and white colobus monkeys (Harris *et al.,* 2009). In addition, the UCP levels of captive chimpanzees, assumed to have greater energy intake than wild animals, were found to be higher than those of their wild con-specifics (Sherry and Ellison, 2007).

Fewer studies have examined variation in UCP level alongside changes in energy expenditure. A recent study (Higham *et al.,* 2011a) investigating the energetics of endurance rivalry among male rhesus macaques demonstrated that UCP levels were negatively associated with time spent travelling and the degree of restlessness during the non-mating season and with various measures of copulation activity during the

mating season. However, no association between UCP level and feeding or resting time was found (Higham *et al.,* 2011a). In addition, the study found that behaviours associated with energy expenditure (travelling time and restlessness) accounted for far more of the variation in BMI than did behaviours associated with energy intake or energy neutral behaviours (i.e. feeding and resting time). These results demonstrate that the influence of energy expenditure on an individual's energetic status can be just as important, if not more so, than the influence of energy intake. Another aspect of energy expenditure, immune defence, resulted in substantially lowered UCP levels in free-ranging chimpanzees during an outbreak of respiratory disease (Emery Thompson *et al.,* 2009).

Table 4.1. Details and summary of results from studies measuring C-peptide concentrations in non-human primates, presented in chronological order. (Continued over page)

Species	Wild/ Captive	Sex (number of individuals)	Serum or urine	If urine, storage method	Relationship between C-peptide and energetic status measures	Reference
Rhesus macaques	Captive	Obese: 4 male, 3 female Non-obese: 3 male, 2 female	Urine	Frozen	Higher in obese compared to non-obese subjects. Decreased during food deprivation in both groups.	Wolden-Hanson *et al.*, 1993
Chimpanzee	Captive	Male (14) Female (15)	Serum	N/A	+ve correlation with plasma C-peptides.	Sherry and Ellison, 2007
Chimpanzee	Captive	Male (14) Female (15)	Urine	Frozen	Higher in captive than in wild.	Sherry and Ellison, 2007
Chimpanzee	Wild (Kanyawara community)	Male (6) Female (6)	Urine	Frozen	+vely related to fruit abundance (only males). During high fruit period, higher in higher ranking males.	Sherry and Ellison, 2007
Bornea orangutan	Wild (Gunung Palung National Park)	Sex not specified (3)	Urine	Filter paper	+vely related to fruit abundance.	Sherry and Ellison, 2007
Bornean orangutan	Wild (Gunung Palung National Park)	Males (5) Females (6)	Urine	Filter paper	-vely related to presence of ketones. Monthly averages +vely related to monthly food availability and intake.	Emery Thompson and Knott, 2008
Baboon (subspecies not specified)	Captive	Male (10) Female (10	Serum	N/A	N/A	Chavez *et al.*, 2008
Bonobo	Captive	Males: 1 adult, 1 juvenile, 1infant Females: 6 adults, 1 juvenile	Urine	Frozen	+vely related to food availability and increases in body mass. (2 pregnant females and 1 infant did not lose body mass and so excluded from most analyses).	Deschner *et al.*, 2008
Chimpanzee	Wild (Kanyawara community)	Males (13)	Urine	Frozen	+ve correlation with amount of fruit and preferred fruit in diet. Low levels during disease outbreak despite food abundance. Higher levels in community occupying a more productive habitat. Higher levels amongst low ranking males.	Emery Thompson *et al.*, 2009

Table 4.1. Continued.

Species	Wild/ Captive	Sex (number of individuals)	Serum or urine	If urine, storage method	Relationship between C-peptide and energetic status measures	Reference
Black and white colobus	Wild	Female, Lactating (2)	Urine	Frozen	+vely related to availability of preferred foods.	Harris *et al.*, 2009
Bonobo	Wild	Females (n=unknown, urine samples not attributed to individual animals)	Urine	Filter paper	Higher when preferred food (ripe fruit) was available.	Georgiev *et al.*, 2011
Rhesus (4) and long-tailed (7) macaques	Captive	Males (8) Females (3)	Urine	Frozen	+ve correlations with plasma C-peptides, skinfold-fatness and BMI. Co-varied with food availability and body mass.	Girard-Buttoz *et al.*, 2011
Rhesus macaques	Free-ranging, provisioned (Cayo Santiago)	Males (13)	Urine	Frozen	+ve correlations with skinfold-fatness and BMI.	Girard-Buttoz *et al.*, 2011
Rhesus macaques	Free-ranging, provisioned (Cayo Santiago)	Males (35)	Urine	Frozen	-vely correlated with travelling time, restlessness and copulation behaviours.	Higham *et al.*, 2011a
Olive baboons	Wild	Female, Wild-feeding (11) Food-enhanced (5)	Urine	Filter paper	+vely correlated with fruit index, rainfall and temperature. -vely correlated with monthly energy expenditure.	Present study

Effect of rank

Several of the studies mentioned above have also investigated how UCPs vary with an individual's position in a dominance hierarchy and the results have been mixed. In preliminary data from the Kanyawara chimpanzee community the two highest ranking males exhibited significantly higher UCP levels than the two lowest ranking males during one season of high fruit abundance (Sherry and Ellison, 2007). However, when a larger data set was utilised low ranking males exhibited significantly higher UCP levels than high ranking males, an effect attributed to costs associated with dominance (Emery Thompson *et al.,* 2009). In male rhesus macaques UCP levels were positively associated with rank during the birthing season but negatively associated with rank by the end of the mating season, apparently due to the increased costs of mating behaviour experienced by the higher ranking males (Higham *et al.*, 2011a).

The effect of female rank and female reproductive state on UCP levels has not yet been investigated in any non-human primate species. In general, more studies have focussed on male rather than female animals, in part due to the complications added to the interpretation of results which include pregnant and lactating animals, since the influence of reproductive state on individual energy balance is not yet fully understood (Sherry and Ellison, 2007; Emery Thompson *et al.*, 2009)

Urine sample storage methods

The storage of biological samples can often be a major challenge in studies involving long periods of field work, especially in remote locations, where equipment and electricity may be limited or unreliable (Higham *et al.,* 2011b). Most of the studies described in section 4.1.1 have stored urine samples by freezing. However, all the samples from orangutans were stored dried on filter paper rather than being frozen

(Sherry and Ellison, 2007; Emery Thompson and Knott, 2008; Emery Thompson *et al.,* 2009). Two validations of this technique have been performed with human urine samples whereby sub-samples were dried on filter paper, using identical procedures to those used in the field to store orangutan urine samples. The C-peptide concentration of the urine extracted from the filter paper was then compared to the C-peptide concentration in the original thawed urine sample (Sherry and Ellison 2007; Emery Thompson and Knott, 2008). In both cases a significant and strong, positive correlation was found between the C-peptide content of the filter paper sample and the original sample. However, although Sherry and Ellison (2007) found no significant difference in the magnitude of the UCP values in their sample of 12 matched filter paper and frozen samples, Emery Thompson and Knott (2008) found that the UCP content of their 22 filter paper samples was significantly lower than their equivalent frozen sample, representing around three quarters of the original, frozen sample. Similar results were obtained in a filter paper storage validation experiment with urine sampled from captive rhesus and long-tailed macaques (Higham *et al.,* 2011b). UCP values of sub-samples stored on filter paper were around 50% of matched samples stored frozen but the values did correlate strongly (Higham *et al.,* 2011b). A study on wild female bonobos has also used the filter paper storage method to preserve urine samples and was able to demonstrate an effect of food quality on UCP level, although this storage technique was not validated as part of the study (Georgiev *et al.*, 2011).

Significance of the current study

The use of UCPs to quantify energetic status in free-ranging non-human primates is still in its infancy. No study has yet attempted an investigation of the effects of female rank and reproductive state on UCP levels and, although the relationship between food availability has been assessed for several species (table 4.1), no study has investigated

the relationship between UCP levels and weather variables, which can influence time budgets (Dunbar, 1992), food availability (Toledo *et al.*, 2011) and disease risk (Nunn *et al.*, 2006). In addition, no study has yet investigated UCP levels in wild baboons and, given the wide geographic and ecological spread of this species and how intensively most aspects of its life have been studied (Jolly, 2007), a study of UCPs in this species seems particularly pertinent.

4.1.3. Predictions

In this chapter, data on the UCP levels of the Gashaka baboons are presented. Following on from the analyses of the calculated energetic status measures in chapter 3, the effect of troop, season, rank and reproductive state on UCP level are assessed, as are the relationships between UCP level and the weather and fruit index variables. In addition, the relationships between UCP level and the calculated energetic measures (intake and expenditure rate) are assessed. The following predictions were developed from the three study hypotheses:

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

Prediction: Gamgam troop will have higher UCP levels than Kwano troop.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary

Predictions:

a. Pregnant or lactating females will have lower UCP levels than cycling females.
b. Low ranking animals will have lower UCP levels than high ranking animals.
c. UCPs will correlate positively with calculated energy intake rate.

d. UCPs will correlate negatively with calculated energy expenditure rate.

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability.

Prediction: UCPs will correlate positively with food availability, rainfall and temperature.

During any study of this kind it is important to remember that UCPs do not simply represent an instantaneous snapshot of an individual's energy balance, in terms of whether an animal has eaten well and exercised little on a particular day, but are also dependent on an individual's previous condition, such as whether an animal is recovering from a period of low energy intake (Deschner *et al.*, 2008). In this way they represent not only an individual's daily energy balance, but also that individual's overall energetic status which incorporates an animal's energy stores (i.e. fat deposits), which are dependent on previous as well as current energy balance. For example, Girard-Buttoz *et al.* (2011) found that when food availability was returned to normal levels following a period of food restriction, the UCP levels of macaques increased day by day, alongside increasing body mass, even through food availability, and therefore presumably energy intake, remained stable. For this reason, the relationship between UCP levels and measures of energy intake or expenditure will not always be simple.

4.2. Results

4.2.1. Overview of UCP data and methodological analyses

A total of 92 UCP values meeting the criteria described in chapter 2 are used in the following analyses. The data range from 1.38 to 226.05 ng UCP /mg creatinine and are

heavily right skewed. Table 4.2 shows the number of samples, and median UCP values for each individual and for the two troops.

Table 4.2. Median and range of UCP concentrations for individual focal animals and troops

FocalID	Median UCP concentration[1] (range in brackets)	n
BRA	6.82 (54.12)	7
DRK	6.78 (31.09)	6
FDI	21.00 (81.18)	4
KRM	31.09	1
KYE	19.88 (47.97)	4
LDI	11.82 (17.25)	3
LMI	4.86	1
MOM	6.08 (41.45)	10
SDY	3.57 (12.60)	4
TOJ	5.07 (17.12)	3
YMK	15.93 (25.50)	6
Kwano	**9.07 (89.57)**	**49**
BUD	17.76 (25.27)	5
KNE	50.14 (223.67)	13
MMK	5.73 (73.75)	10
MMW	19.07 (20.62)	4
STR	8.17 (51.12)	11
Gamgam	**13.46 (224.67)**	**43**
Gamgam without KNE, pregnant values[3]	**10.18 (73.76)**	**36**
Both troops	**10.84 (224.67)**	**92**
Both troops without KNE, pregnant values[3]	**9.74 (89.8)**	**85**

1. UCP concentration in ng/mg creatinine
2. Troop values are medians from all UCP values for that troop
3. Troop and total medians are presented with and without 7 unusually high values from samples collected from Gamgam female, KNE when she was pregnant (see section below for discussion).

One individual from Gamgam troop, KNE, exhibited particularly high UCP levels, with a median UCP level almost 5 times greater than the median of the entire dataset. Figure 4.2, shows her UCP results for the entire study period. KNE exhibited particularly high levels when she was pregnant but during lactation her UCP values dropped to around the overall median level of the two troops. Possible reasons for this are discussed in section 4.3.3 but for all further analyses these seven KNE pregnant values are excluded.

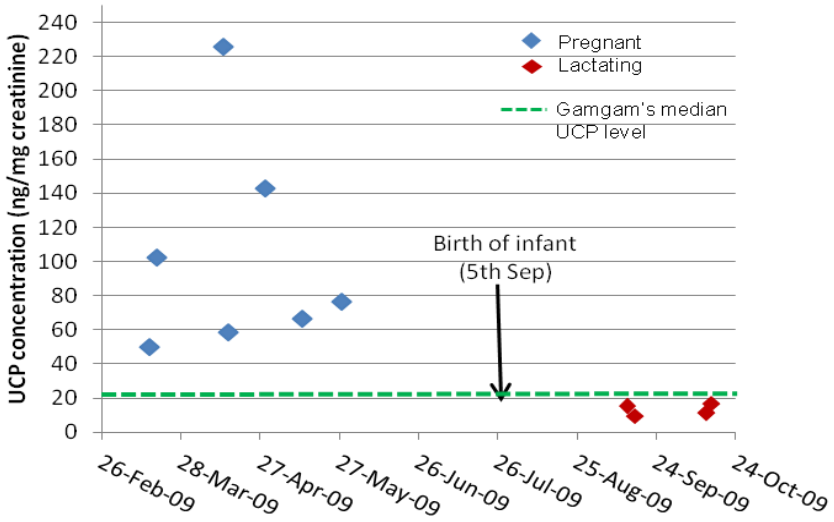

Figure 4.2. Scatter plot showing UCP levels for Gamgam female, KNE throughout study period.

C-peptide clearance and excretion time

In humans, C-peptide has a clearance time of around 1 ½ to 2 hours from the blood (Kjems *et al.*, 2000) and the time lag to excretion of hormones in urine is usually 4-8 hours in non-human primates (Hodges and Heistermann, 2003). While C-peptide levels measured in a blood samples provide an instantaneous snapshot of current, circulating levels, urinary C-peptide provides an integrated measure of circulating levels over the time between successive urinations (Cook and Beastall, 1987). C-peptide levels measured in urine may therefore reflect the energetic status/ energy balance of an individual around 6-10 hours prior to collection of the sample. Given that this period is less than 24hrs, the UCP data collected for the current study will be matched up with other data (e.g. rainfall, focal observation data) collected on the same day as the urine sample rather than the previous day.

Repeated analysis of urine samples

For 12 of the 92 samples enough urine was obtained to store sufficient quantities for analysis on two different protein saver cards. These sub-samples were analysed

separately for UCP content, allowing the reliability of the procedure to be assessed (table 4.3).

Table 4.3. UCP concentrations (ng/mg creatinine) from repeated analysis of urine samples

Sample	Sub-sample 1	Sub-sample 2	% deviation between sub-samples
1	3.78	4.50	16
2	9.85	8.30	16
3	12.66	7.35	42
4	4.61	5.07	9
5	29.79	32.39	8
6	8.84	14.81	40
7	3.86	3.69	4
8	9.69	6.13	37
9	6.08	2.36	61
10	7.38	2.76	63
11	37.64	19.18	49
12	36.99	25.48	31
	Mean % deviation ± s.e.		**31±6**

The % deviation between sub-samples varied from 4-63% with a mean of 31%. There is a significant and strong, positive relationship between the C-peptide concentrations of the 12 repeated samples (Spearman's rank correlation, r_s=0.811, n=12, p=0.001, figure 4.3).

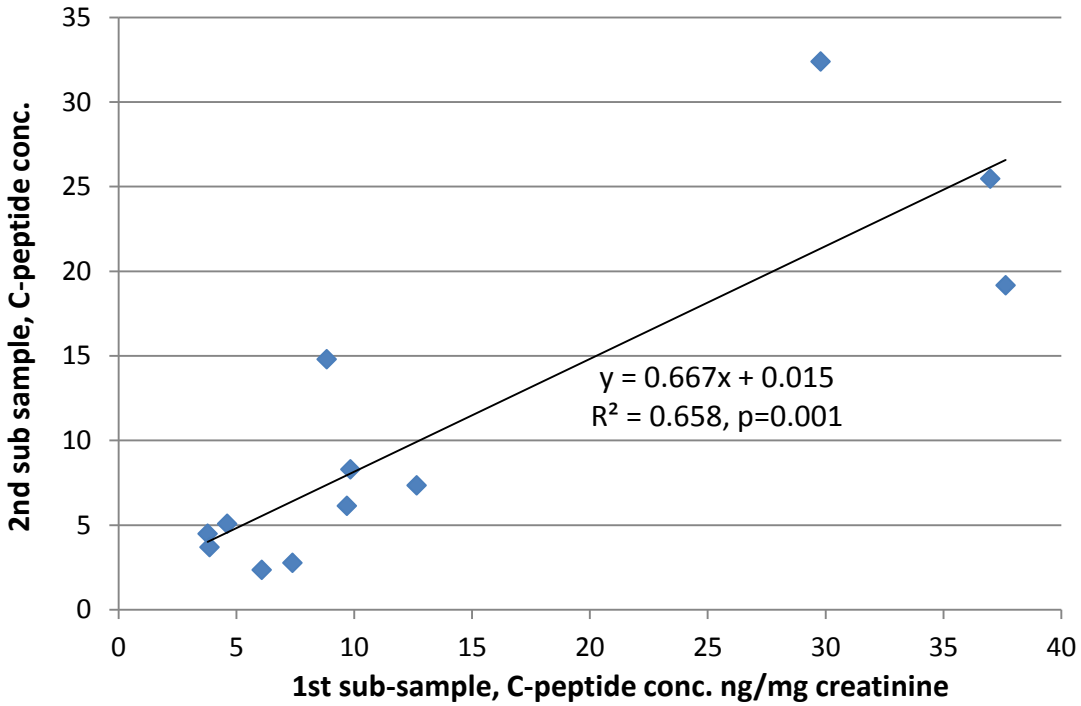

Figure 4.3. Scatter plot showing correlation between the C-peptide concentrations of sub-samples of the same urine sample with best fit line.

Effect of collection time on UCP levels

The effect of collection time on UCP level was assessed by comparing values collected at different hours of the day (e.g. between 06:00-06:59 and 07:00-07:59) and during the morning (06:44 - 11:59) and afternoon (12:00 - 18:02). Hour of collection had no significant effect on UCP value (Kruskal-Wallis: χ^2=10.19, d.f.=9, p=0.336; figure 4.4) and morning samples did not have significantly different UCP values from afternoon samples (Mann-Whitney U test: U=799.5, n=51,34, p=0.545). There was also no significant difference between the UCP values of samples collected in the early morning (before 7:00 or 8:00), which may represent fasting levels, and those collected during the rest of the day (Mann-Whitney U test: samples collected before 07:00, U=294, n before 07:00=8, n after 07:00=77, p=0.833; samples collected before 08:00, U=435, n before 08:00=12, n after 08:00=73, p=0.970).

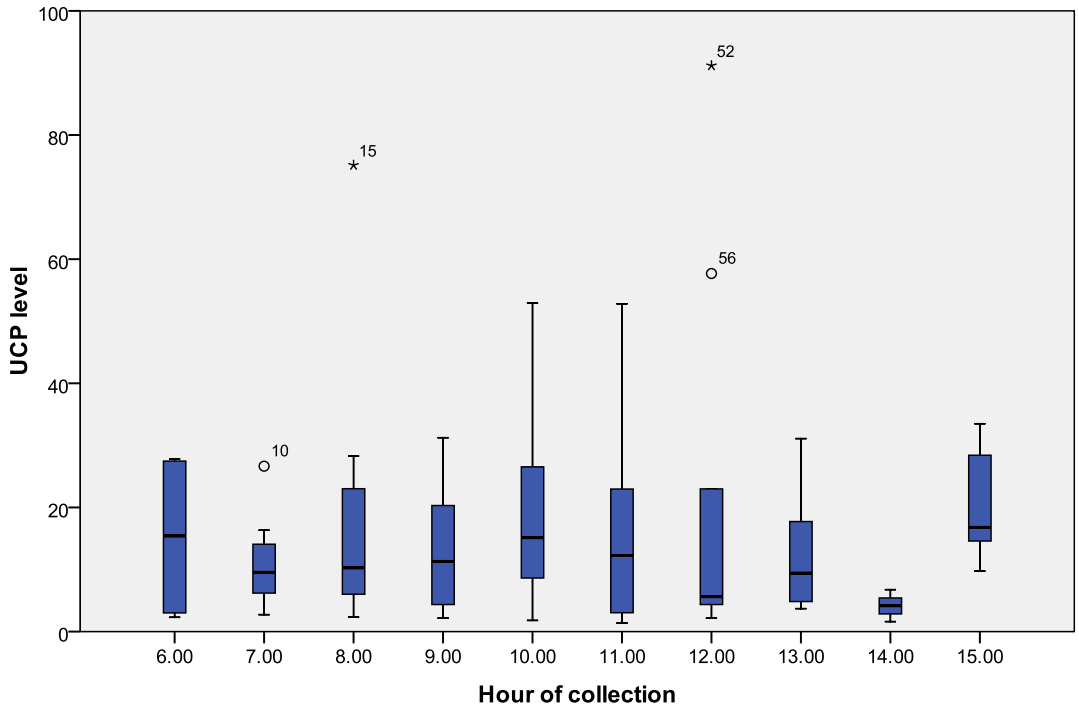

Figure 4.4. Box-plot showing UCP levels of samples collected at different hours of the day. Samples collected between 15:00 and 18:02 were pooled into one group for these analyses due to the small number of samples collected within these hours (n=6).

4.2.2. Variation in UCP level between different troops, seasons, ranks and reproductive states

The effects of the categorical variables (troop, season, rank and reproductive state) on UCP level were assessed by building GLMMs with Log_{10} transformed UCP data (n=85 i.e. with 7 KNE pregnant values removed) as the dependent variable, and urine sample number and ID fitted as random effects. The categorical variables troop, season, rank and reproductive state were then added to the model in turn as fixed effects. All 2-way interactions between the categorical variables were also investigated. Full results of statistical analyses relating to all the models in this chapter are presented in appendix 6b.

Neither the addition of troop (D=0.046, d.f.=1, p=0.830) nor reproductive state (D=1.94, d.f.=2, p=0.379) significantly improved the fit of the UCP 2-factor null model

(figure 4.5a and d). There was, however, a marginally non-significant increase in the explanatory power of the null model on the addition of season (D=76.92, d.f.=1, p=0.062), with a trend for higher UCP levels during the wet season than during the dry season (z=1.88, p=0.06, figure 4.5b) and a significant increase in explanatory power on the addition of rank (D=6.44, d.f.=2, p=0.04), with low ranking animals exhibiting higher UCP levels than both high (z=2.54, p=0.011) and middle ranking (z=1.85, p=0.065) animals (figure 4.5c).

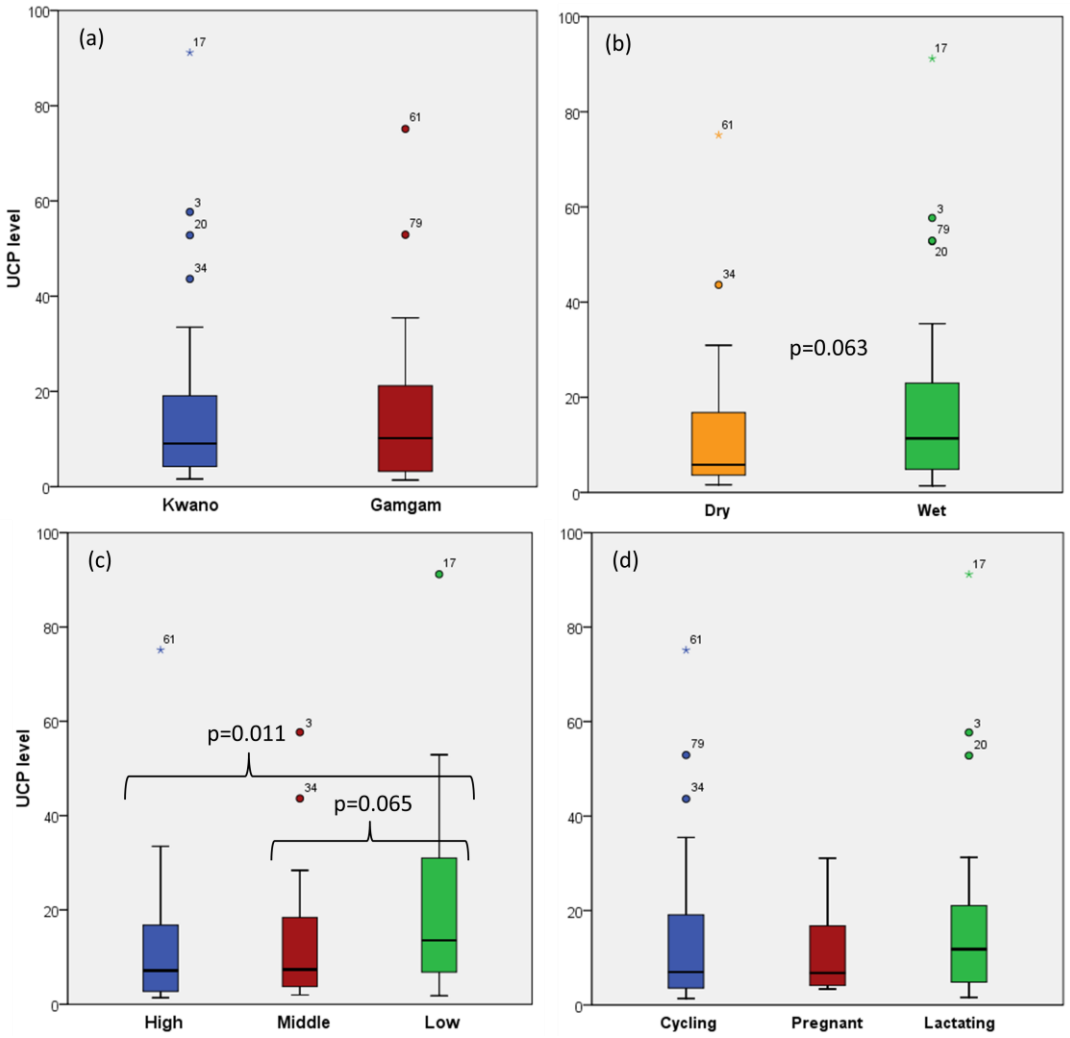

Figure 4.5. Box-plots showing UCP levels (ng/mg creatinine) of samples collected from individuals from different troops (a), during different seasons (b), of different ranks (c) and in different reproductive states (d).

There was a marginally non-significant interaction effect between season and troop (D=2.98, d.f.=1, p=0.084), with significantly higher UCP levels during the wet season than during the dry season for Kwano troop (z=2.61, p=0.009) but not for Gamgam troop (z=0.07, p=0.943). UCP levels did not differ significantly between troops during either season (dry: z=1.02, p=0.310; wet: 1.46, p=0.143) (figure 4.6). No other interactions between the categorical variables were significant (appendix 6b, table A6.vi).

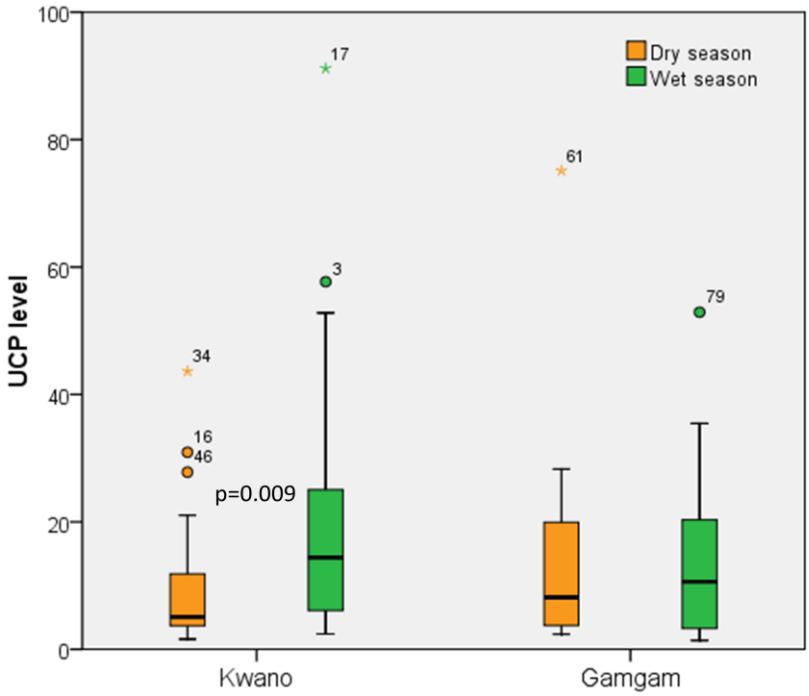

Figure 4.6. Box-plot showing the variation in the UCP levels (ng/mg creatinine) of samples collated during different seasons and from different troops.

4.2.3. Comparison of UCPs with weather and fruit index variables

The relationships between UCP level and the weather and fruit index variables were assessed in the same way as the relationships between these measures and the calculated energy rates assessed in chapter 3, by adding each of the variables to the UCP 2-factor null model, in turn, as fixed effects.

The addition of both daily and 30-day rainfall to the UCP 2-factor null model significantly improved its fit (daily rain: D=6.17, d.f.=1, p=0.013; 30-day rain: D=6.84, p=0.009), with significant positive relationships between UCP level and both the rainfall variables (daily rain: z=2.53, p=0.011, figure 4.7a; 30-day rain: z=2.67, p=0.008, figure 4.7b).

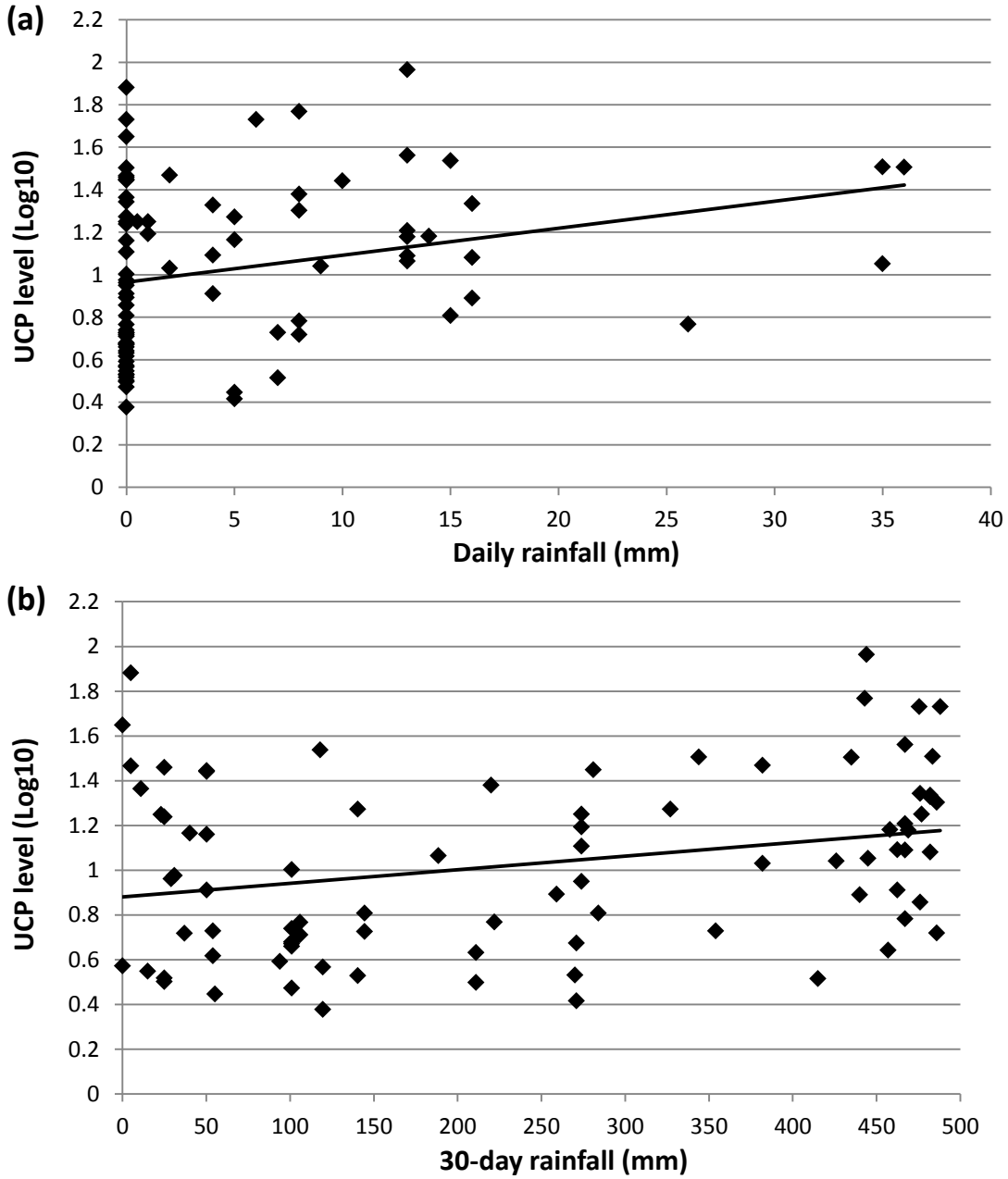

Figure 4.7. Scatter plots showing the relationship between UCP level (ng/mg creatinine) and both (a) daily rainfall and (b) 30-day rainfall. The lines represent the average linear relationships between the two variables for all individuals, as predicted by the GLMM.

The addition of tree-fruit index to the 2-factor UCP null model significantly improved its fit (D=7.08, d.f.=1, p=0.008), with a significant positive correlation between the two variables (z=2.72, p=0.007, figure 4.8).

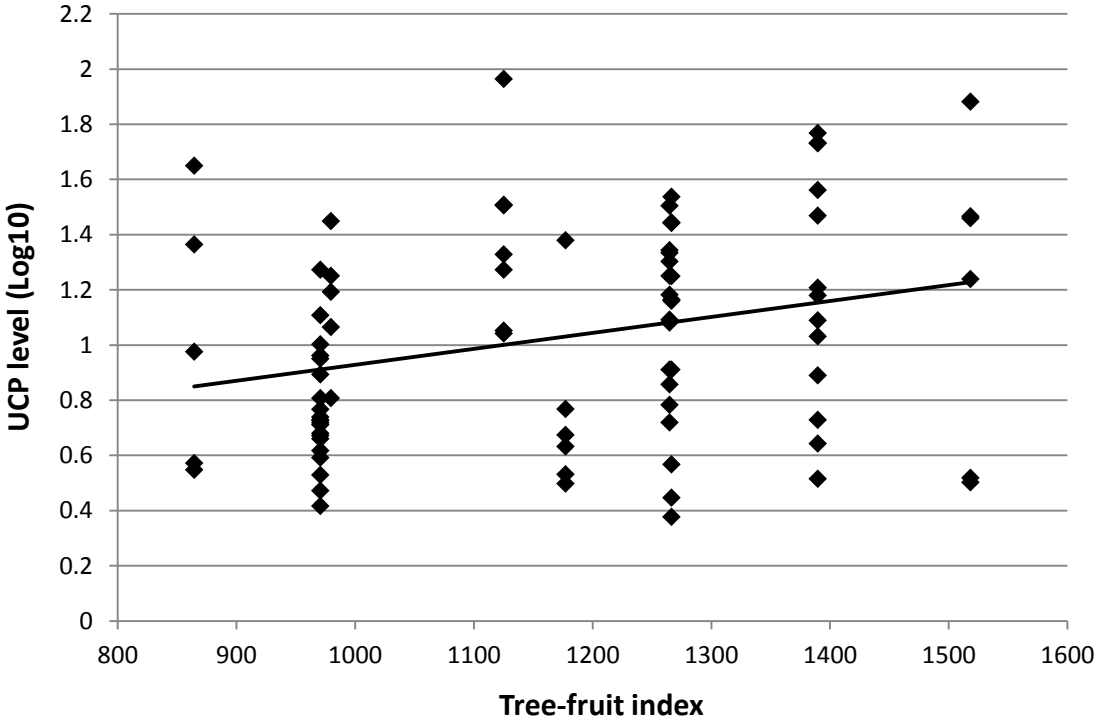

Figure 4.8. Scatter plot showing the relationship between UCP level (ng/mg creatinine) and tree-fruit index. The line represents the average linear relationships between the two variables for all individuals, as predicted by the GLMM.

Tree-fruit index correlates significantly and positively with both daily and 30-day rainfall (Spearman's rank correlation, n=85: daily: r_s=0.30, p=0.006; 30-day: r_s=0.25, p=0.022) so in order to determine whether the effect of tree-fruit index on UCP level was influenced by rainfall and vice versa, UCP models were built containing both tree-fruit index and either daily or 30-day rainfall. These models were then compared to the UCP model containing just tree-fruit index, since the addition of tree-fruit index improved the fit of the UCP 2-factor null model slightly more than the addition of either rainfall variable.

The addition of either rainfall variable to the UCP model containing tree-fruit index improved the fit of the model, although the effect was marginally non-significant for 30-day rainfall (daily: $D=4.80$, d.f.=1, $p=0.028$; 30-day: $D=3.65$, d.f.=1, $p=0.056$). For both models (one containing daily rainfall and one containing 30-day rainfall), the positive relationship between rainfall and UCP level remained significant after controlling for the effect of tree-fruit index (daily: $z=2.22$, $p=0.026$; 30-day: $z=1.99$, $p=0.046$). For the model containing daily rainfall, the positive relationship between tree-fruit index and UCP level remained significant after controlling for the effect of rainfall ($z=2.42$, $p=0.015$). For the model containing 30-day rainfall, the positive relationship between tree-fruit index and UCP level became marginally-non significant after controlling for the effect of rainfall ($z=1.93$, $p=0.054$).

Interactions between categorical variables and the weather and fruit index variables

The categorical variables (troop, season, rank and reproductive state) were fitted into UCP models alongside the weather and fruit index variables in order to investigate whether the relationships with UCP levels differ between individuals belonging to different troops, ranks and reproductive states and during different seasons.

The addition of the season interaction effect to the weather and fruit index models improved the fit of five out of the nine models. This effect was highly significant for daily and monthly maximum temperature (daily: $D=3.09$, d.f.=1, $p<0.001$; monthly: $D=12.10$, d.f.=1, $p=0.001$), with a significant positive relationship between UCP level and maximum temperature during the dry season (daily: $z=3.87$, $p=0.002$; monthly: $z=2.92$, $p=0.004$) and a significant negative relationship during the wet season (daily: $z=3.87$, $p<0.001$; monthly: $z=2.29$, $p=0.012$) (figure 4.9).

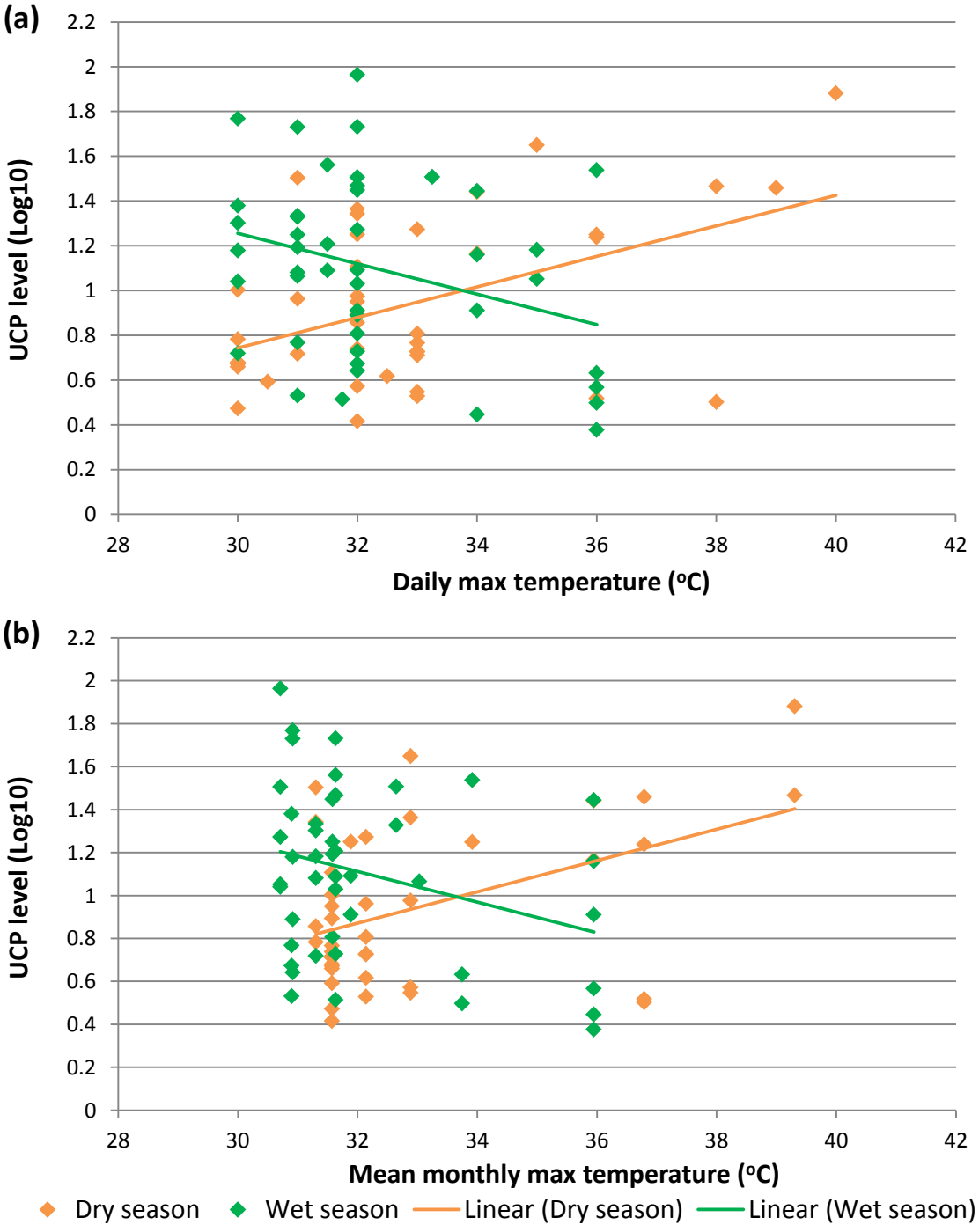

Figure 4.9. Scatter plot showing the effect of season on the relationship between UCP level (ng/mg creatinine) and both (a) daily and (b) mean monthly maximum temperature. The lines represent the average linear relationships between the two variables for all individuals during each season.

The season effect was also highly significant for total and vine-fruit index (total: D=12.99, d.f.=1, p<0.001; vine: D=11.51, d.f.=1, p=0.001), with a significant positive relationship between UCP level and fruit index during the dry season (total: z=2.77,

p=0.006; vine: z=2.66, p=0.008) and a significant negative relationship between the two variables during the wet season (total: z=2.51, p=0.012; vine: z=2.36, p=0.018) (figure 4.10).

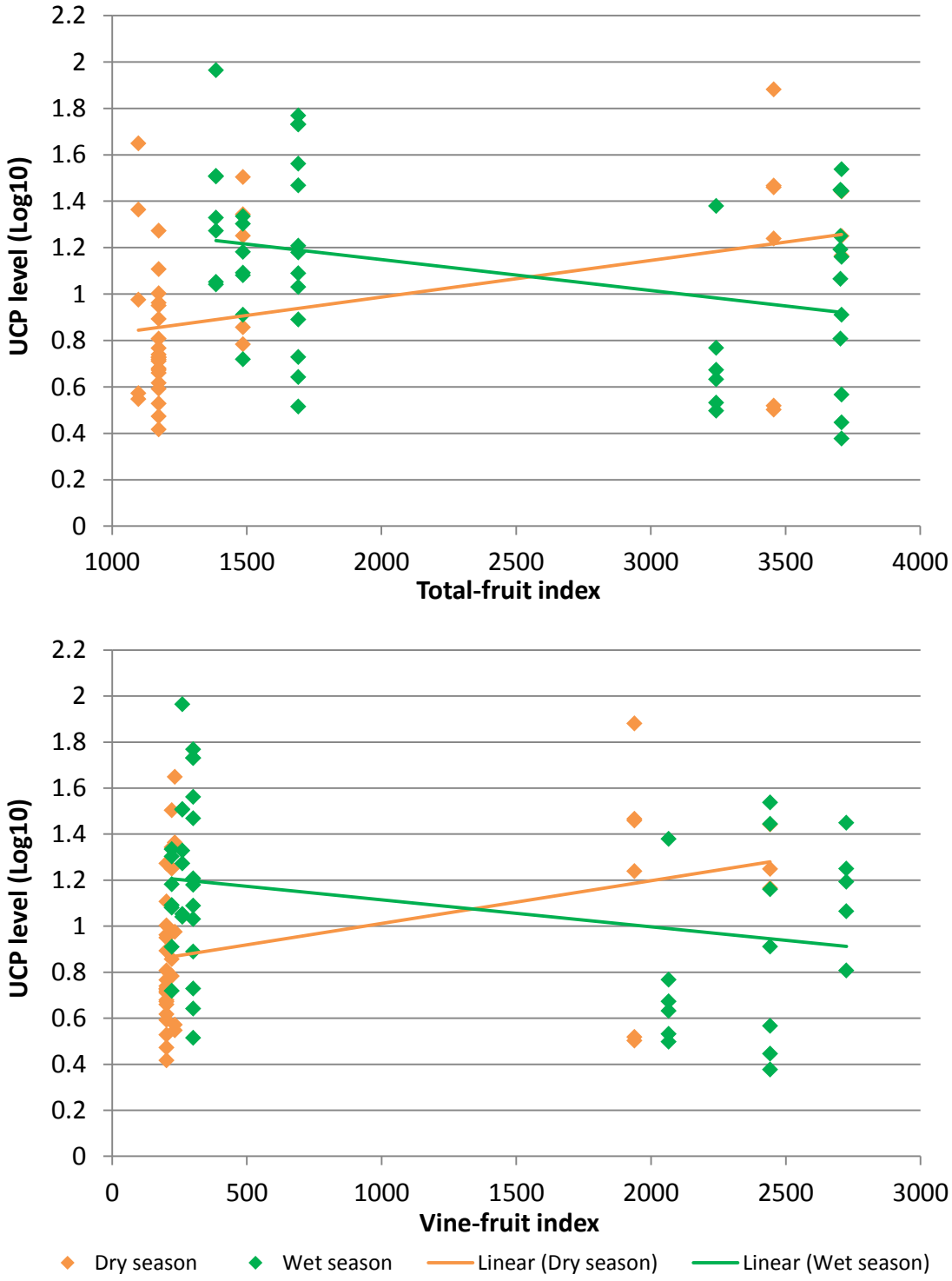

Figure 4.10. Scatter plot showing the effect of season on the relationship between UCP level (ng/mg creatinine) and both (a) total and (b) vine fruit index. The lines represent

the average linear relationships between the two variables for all individuals during each season.

The maximum temperature measures correlate significantly and positively with both vine and total-fruit index (table 4.4.) so in order to determine whether the effect of fruit index on UCP level was influenced by maximum temperature and vice versa, a UCP model was built containing the season interaction effect and the two most closely correlated of these variables: mean monthly maximum temperature and vine-fruit index. This model was compared to the UCP model containing mean monthly maximum temperature, season and their interaction, since this model was slightly better fitting than the equivalent one containing vine-fruit index.

Table 4.4. Results of Spearman's rank correlations between both daily and mean monthly maximum temperature and both vine- and total-fruit index (n=85 for all correlations)

Correlation between variables:	r_s	p
Daily max temperature vs. Vine-fruit index	0.308	0.004
Daily max temperature vs. Total-fruit index	0.304	0.005
Mean monthly max temperature vs. Vine-fruit index	0.331	0.002
Mean monthly max temperature vs. Total-fruit index	0.316	0.003

The addition of vine-fruit index to the UCP model containing mean monthly maximum temperature did not significantly improve the fit of the model (D=1.65, d.f.=2, p=0.439). When the effect of vine-fruit index was controlled for the previously significant relationships between mean monthly maximum temperature and UCP during the dry and wet seasons disappeared (dry: z=1.25, p=0.211; wet: z=1.12 p=0.261). Similarly, when the effect of mean monthly maximum temperature was controlled for the previously significant relationships between vine-fruit index and UCP during the dry and wet seasons disappeared (dry: z=0.17, p=0.864; wet: z=1.28 p=0.201).

The addition of the season interaction effect to the UCP model containing mean monthly minimum temperature marginally non-significantly improved its fit (D=3.47,

d.f.=1, p=0.063) but the relationship between the two variables was not significant in either season (dry season: z=1.50, p=0.134; wet season: z=1.48, p=0.138).

There was no significant improvement in the fit of the UCP models containing any other of the weather and fruit index variables, on addition of the season interaction effect, and the addition of the troop, rank or reproductive state interaction effects did not significantly improve the fit of any of these models (appendix 6b, table A6.iv).

4.2.4. Relationship between UCP level and calculated energy rates

The relationship between UCP level and the calculated energy rates was assessed in two ways: first a direct comparison of UCP values with paired calculated energy balance, intake and expenditure scores from the same day and individual (data available for 47/85 UCP samples), and second by comparing average values of the calculated energy measures for each month and troop with the entire UCP dataset (n=85). In both cases GLMMs were constructed with Log_{10} transformed UCP data as the dependent variable and with sample number and ID fitted as random effects. The categorical variables troop, season, reproductive state and rank were also added to each of the models in turn.

Direct comparison of individual daily UCP values and calculated energy measures

The fit of the UCP 2-factor null model did not significantly improve with addition of either energy intake rate (D=0.08, d.f.=1, p=0.773) or energy expenditure rate (D=0.43, d.f=1, p=0.513). The addition of the rank interaction effect to the UCP model containing energy intake rate significantly improved the model's fit (D=8.63, d.f.=2, p=0.013), with a significant negative relationship between intake rate and UCP level for high ranking animals (z=2.19, p=0.029), a significant positive relationship for middle

ranking animals (z=2.14, p=0.032), and no relationship for low ranking animals (z=0.29, p=0.775; figure 4.11).

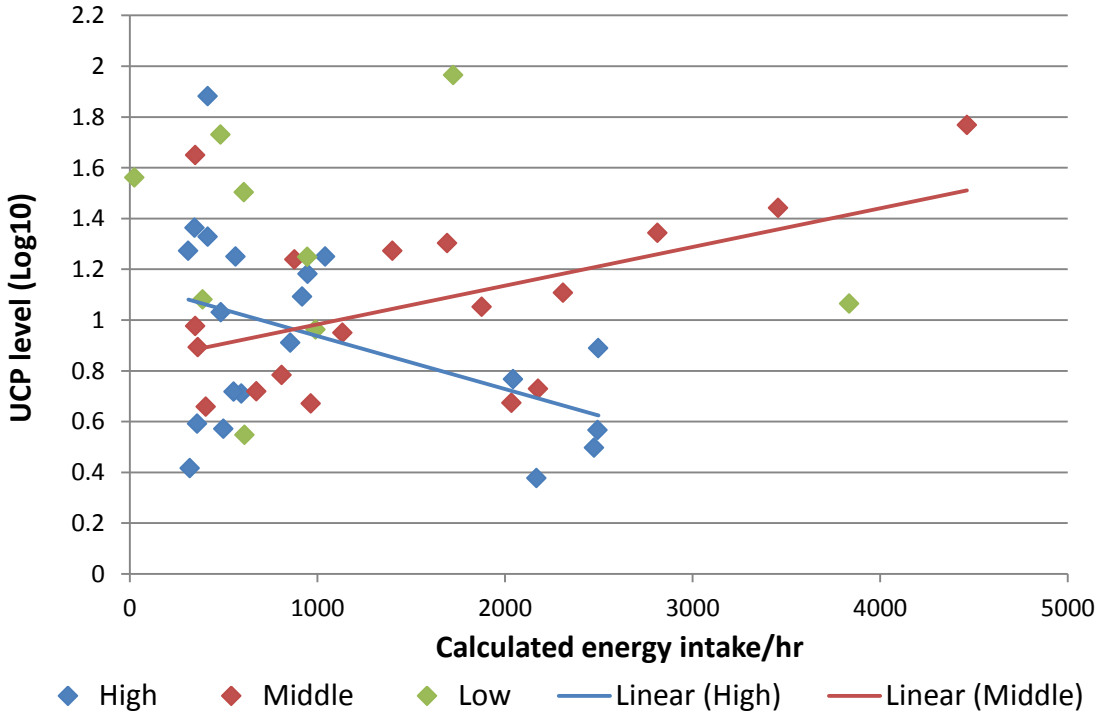

Figure 4.11. Scatter plot showing the effect of rank on the relationship between UCP level (ng/mg creatinine) and calculated energy intake rate (kJ/hr). The lines represent the average linear relationships between the two variables for all individuals from each rank category.

The addition of the reproductive state interaction effect to the UCP model containing energy intake rate resulted in a marginally non-significant improvement in the model's fit (D=5.73, d.f.=2, p=0.057). There was a trend towards a negative relationship between intake rate and UCP level for cycling animals (z=1.80, p=0.072), a trend towards a positive relationship for lactating animals (z=1.69, p=0.091) and no sign of a relationship for pregnant animals (z=0.08, p=0.940; figure 4.12).

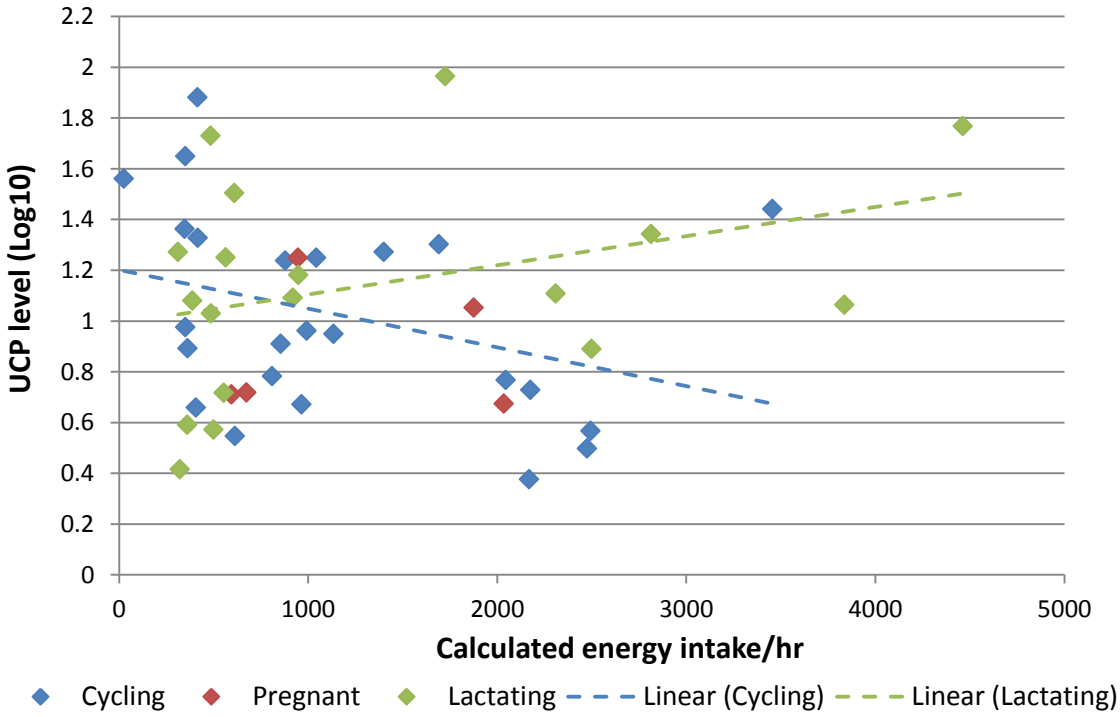

Figure 4.12. Scatter plot showing the effect of reproductive state on the relationship between UCP level (ng/mg creatinine) and calculated energy intake rate (kJ/hr). The lines represent the average linear relationships (marginally non-significant, as indicated by the dashed lined) between the two variables for all individuals that were either cycling or lactating.

The addition of the troop and season interactions did not significantly improve the fit of UCP model containing energy intake rate and none of the categorical interaction variables improved the fit of the UCP model containing energy expenditure rate (appendix 6b, table A6.viii).

Comparison of UCP values with average monthly calculated energy measures

The calculated energy intake and expenditure rate data were right skewed so median rather than mean values were used for the comparison with UCP levels. Median values were calculated for each troop for each month, resulting in a total of 18 'troop-month' values for each of the five calculated energy variables (table 4.5).

Table 4.5. Median calculated energy intake and expenditure rates (kJ) for each troop and month. Values used in GLMMs built to investigate the relationship between UCP levels and monthly calculated energy rates.

	Month	Energy Intake/hr	Energy Expenditure/hr	n
Kwano	March	1448.54	540.91	4
	April	2408.28	565.24	2
	May	1106.69	459.05	4
	June	1494.66	441.11	4
	August	387.03	427.00	5
	September	881.65	447.90	5
	October	865.47	493.50	9
	November	547.99	566.21	14
	December	332.93	512.12	2
Gamgam	March	664.59	512.82	4
	April	2585.80	558.77	11
	May	1586.01	492.52	3
	June	2288.90	459.08	2
	August	191.07	428.95	2
	September	921.89	511.60	8
	October	769.63	484.36	3
	November	965.90	525.83	7
	December	479.56	514.52	3

The addition of median monthly energy expenditure rate to the UCP 2-factor null model resulted in a significant increase in the explanatory power of the model (D=4.07, d.f.=1, p=0.044), with a significant negative relationship between UCP level and expenditure rate (z=2.05, p=0.04; figure 4.13)

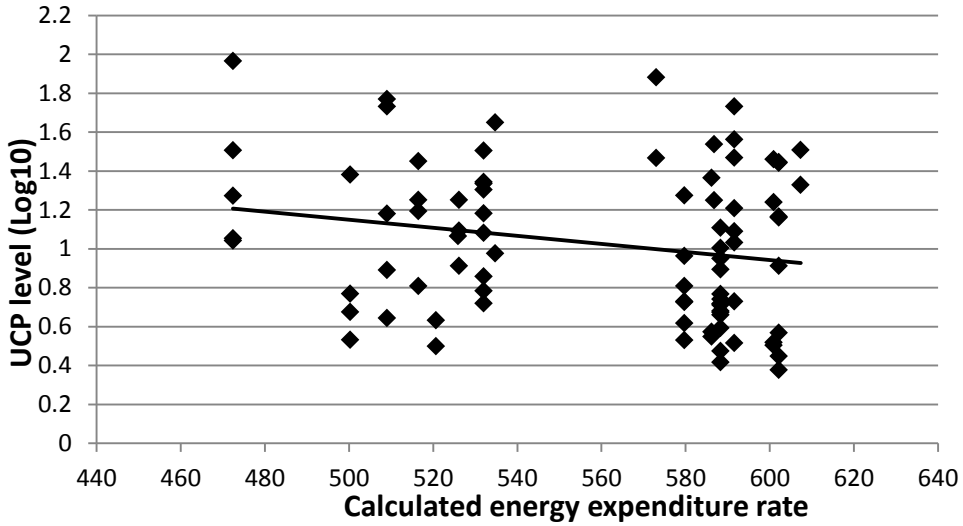

Figure 4.13. Scatter plot showing the relationship between UCP level (ng/mg creatinine) and median monthly calculated energy expenditure rate (kJ/hr). The line represents the average linear relationships between the two variables for all individuals.

The addition of median monthly calculated energy intake rate to the UCP 2-factor null model did not significantly improve its fit (D=0.73, d.f.=1, p=0.394).

Influence of categorical variables on UCP calculated energy measure relationships

The addition of the troop interaction effect significantly improved the fit of the UCP model containing median monthly energy expenditure rate (D=4.56, d.f.=1, p=0.033), with a significant negative relationship between UCP level and expenditure rate for Kwano troop (z=2.99, p=0.003) but no relationship for Gamgam troop (z=0.86, p=0.391) (figure 4.14).

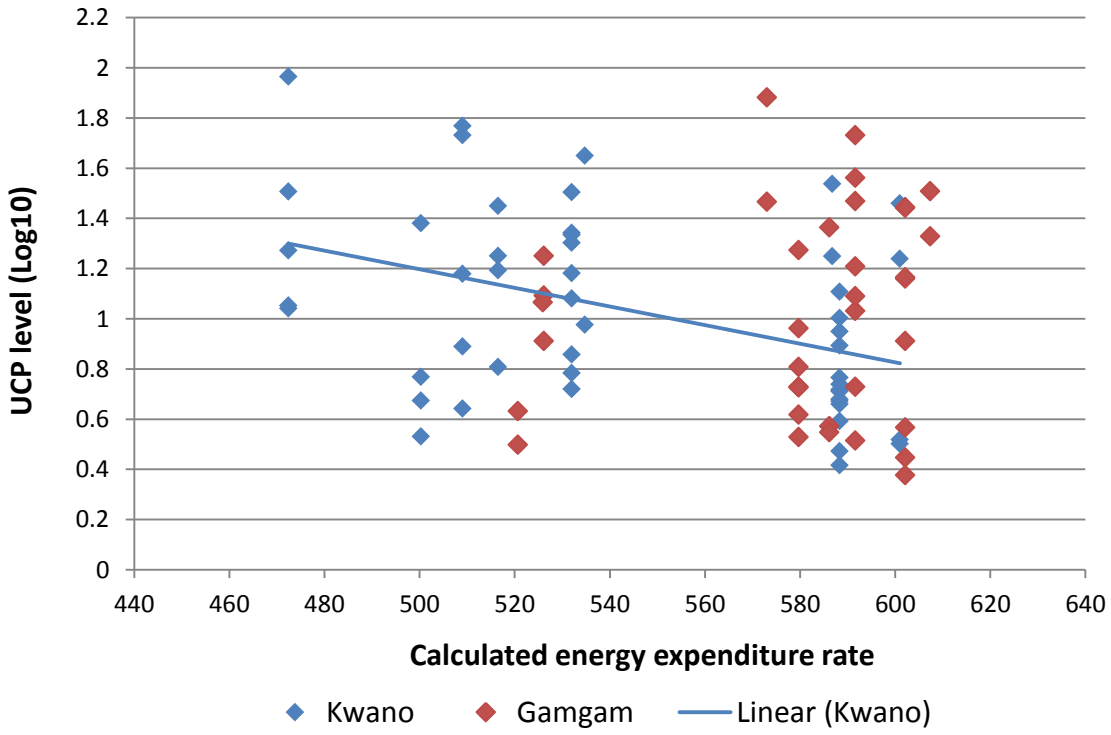

Figure 4.14. Scatter plot showing the effect of troop on the relationship between UCP level (ng/mg creatinine) and median monthly calculated energy expenditure rate (kJ/hr). The lines represent the average linear relationships between the two variables for all individuals in each troop.

The addition of the season interaction effect significantly improved the fit of the UCP model containing median monthly calculated energy intake rate (D=9.31, d.f.=1, p=0.002), with a significant negative relationship between UCP level and energy intake rate during the wet season (3.14, p=0.002) and no relationship during the dry season (z=1.57, p=0.117) (figure 4.15). There were no other significant interactions between the energy rates and the categorical variables: troop, season, rank and reproductive state (appendix 6b, table A6.viii).

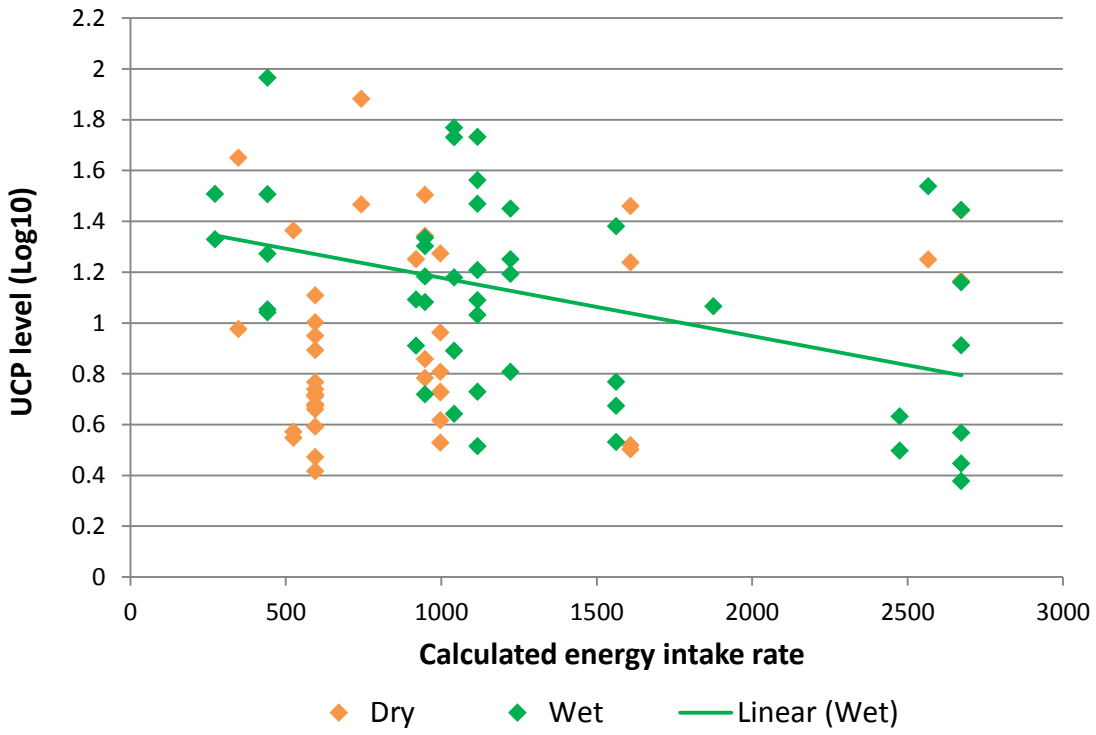

Figure 4.15. Scatter plot showing the effect of season on the relationship between UCP level (ng/mg creatinine) and median monthly calculated energy intake rate (kJ/hr). The lines represent the average linear relationships between the two variables for all individuals during each season.

4.2.5. Effect of Gamgam troop's crop-raiding behaviour

Out of the 36 UCP values for Gamgam troop four were collected on days when the baboons were observed eating crops (crop-feeding days). A comparison of crop-feeding and non-crop-feeding days revealed no significant difference in UCP levels (Mann-Whitney U tests: U=58, n=4,32, p=0.790). A paired t-test comparing the median UCP

levels of individual animals (n=4 because one Gamgam animal, MMW, did not have a UCP value for a crop-raiding day) sampled on days when crop-raiding was and was not observed also found no significant difference (paired t-test: t=-0.422, d.f.=3, p=0.701).

4.3. Discussion

4.3.1. Repeated analysis of urine samples

Previous studies have validated the use of filter paper storage by demonstrating a significant correlation between the UCP content of human urine samples stored frozen and on filter paper (Sherry and Ellison, 2007; Emery Thompson and Knott, 2008). In this study the repeatability of the methods was assessed by comparing the UCP values of 12 samples which had been analysed twice. The sub-samples should contain identical concentrations of UCP so any deviation between them is due to inaccuracies due to the storage, elution or measurement of the sample. Although, substantial deviation was found between the absolute value of repeated samples, the two sets of values were strongly correlated which suggests that the methods used in the current study to measure UCP level techniques can be effective.

4.3.2. Effect of collection time on UCP levels

It is important to address the possibility that UCP levels will vary throughout the day either in predictable patterns, as can be the case with hormones such as testosterone and cortisol (e.g. van Schaik *et al.,* 1991; Muller and Lipson, 2003), or in response to recent feeding. While some previous analyses of UCP in human and non-human primates have emphasised the importance of collecting early morning voids of urine (Ellison and Valeggia, 2003; Sherry and Ellison, 2007), other researchers have found no consistent changes in UCP throughout the day (Deschner *et al.,* 2008; Emery Thompson *et al.,* 2009). For example, in a study of captive bonobos no difference was found between the

C-peptide concentration of urine samples collected after an overnight fast and 4hrs after feeding. In contrast, C-peptide levels of urine samples collected from captive macaques after an overnight fast were, on average, three times lower than samples collected 2-3hrs after their morning feed, although the effects of a diet restriction experiment outweighed this effect (Girard-Buttoz *et al.*, 2011). Due to the practicalities of collection in the current study, urine samples were collected throughout the observation day. However, no consistent effect of collection time was found on the UCP values and there was also no significant difference between samples collected in the very early morning, which may be considered to represent fasting levels, and samples collected during the rest of the day, although the possibility of the baboons feeding during the night cannot be ruled out. These results suggest that collection time will have a minimal effect on UCP level in this study, which is consistent with the idea that short term variations in UCPs are likely to be less in wild animals than in captive animals due to the fact that their feeding is spread out over the course of a day rather being concentrated on a few energy rich meals (Girard-Buttoz *et al.*, 2011).

4.3.3. A possible case of gestational diabetes mellitus

The unusually high UCP levels exhibited by the individual KNE during her pregnancy require an explanation. Several characteristics of these urine samples mean that relatively high UCP levels were to be expected, such as the fact that the samples were collected during a period of high fruit availability and high temperature (both predicted to correlate positively with UCP levels), KNE's high ranking status (high ranking animals were predicted to have higher UCP levels than lower ranking animals) and her membership of the food-enhanced troop (which was predicted to have higher UCP levels than the wild-feeding troop). However, the fact that these UCP values were around five times higher than the average level and the fact that during lactation her

values dropped to around the average level for the two troops suggests another influence. One possibility is that this female experienced gestational diabetes mellitus, a form of insulin resistant, type II, diabetes which develops spontaneously during pregnancy. To the best of my knowledge, the development of gestational diabetes has been confirmed in only one non-human primate, a captive white faced saki (Lloyd *et al*., 1995), but the spontaneous development of type II diabetes has been well documented in several Old World primate species (Wagner *et al.,* 2006) including wild, refuse-raiding baboons (Banks *et al.,* 2003). Although gestational and type II diabetes are not necessarily associated with higher UCP levels they are often associated with hyperinsulinemia, which means higher than normal production of insulin and therefore higher UCP levels are possible (Wagner *et al.,* 2006) and in humans, gestational diabetes has been associated with elevated UCP levels (Nordlander *et al.,* 1989). The presence of gestational diabetes in this individual could, in theory, have been confirmed by testing for glucose in the urine samples (Lloyd *et al*., 1995) but unfortunately all the urine samples were entirely used up for the analysis of C-peptide content.

4.3.4. Effect of weather and food availability on UCP levels

As predicted, UCP levels were higher during the wet season than during the dry season (although this effect was marginally non-significant) and UCP level correlated significantly and positively with both measures of rainfall and with the tree-fruit index. Although rainfall and tree-fruit index were positively correlated, they appear to be independently related to UCP level, since the effect of each of these variables on UCP level remains when the effect of the other is controlled for. These results are consistent with the idea that better energetic status is associated with greater primary productivity (due to higher rainfall) and, subsequently, higher food availability. These results are also consistent with the activity budget and calculated energy rate results, presented in

chapter 2, showing that the wet season was characterised by more time spent resting and in social behaviours, and less time spent feeding and travelling, as well as lower energy expenditure rates and a trend for higher energy intake rates compared to the dry season.

The interaction between season and troop was marginally non-significant, with only Kwano troop exhibiting significantly higher UCP levels in the wet season compared to the dry season. This provides further support for the idea that Gamgam's crop-raiding behaviour buffers it against environmental factors, as suggested for food enhanced baboons at Amboseli (Bronikowski and Altmann, 1996) and is consistent with results from chapter 3 showing a season effect on calculated energy expenditure for Kwano troop but not Gamgam troop.

The predicted positive relationship between UCP level and vine-fruit index (as well as the closely correlated total-fruit index) was only present during the dry season whereas during the wet season there was an unexpected negative relationship. Similarly, the relationships between UCP level and both daily and monthly mean maximum temperature were positive during the dry season but negative during the wet season.

When the effect of vine-fruit index was controlled for, the season-specific significant relationships between mean monthly maximum temperature and UCP level disappeared as did the relationships between UCP level and vine-fruit index when the effect of maximum temperature was controlled for. This suggests that the same factor is driving the relationship between these two variables and UCP level: either vine-fruit index, maximum temperature or a third variable that both variables are related to. The seasonal difference suggests that food availability may be having a strong influence on the Gashaka baboons' energetic status during the dry season but that other factors, such as

disease prevalence, may have a stronger effect during the wet season. For example, high temperatures during the wet season, which are associated with the lowest UCP levels, may represent the optimal conditions for disease prevalence (Nunn *et al.,* 2006), so it may be that the energetic status of the baboons is low during this period due to a high disease burden, despite the high food availability, which is also associated with high temperatures and rainfall.

4.3.5. Effect of troop, reproductive state and rank on UCP levels

Troop

No difference was found between the UCP values of animals belonging to the two different troops. This is not as predicted and suggests that food-enhancement does not have a significant effect on this measure of energetic status in the study animals. This result is in contrast to the activity budget and energy intake rate results presented in chapter 3, which showed that Gamgam troop spent more time resting and less time feeding than Kwano troop and also exhibited a substantially higher energy intake rate alongside an only slightly elevated expenditure rate. No previous study has investigated the impact of food-enhancement on UCP levels of wild-feeding primates. However, captive chimpanzees had higher UCP levels than wild con-specifics (Sherry and Ellison, 2007) and free-ranging baboons, which supplemented their wild diet with raids of tourist refuse sites, exhibit significantly higher serum insulin levels than entirely wild-feeding members of the same population (Kemnitz *et al*., 2002). The effect of the type of food-enhancement experienced at Gashaka, however, is likely to be far more subtle than the difference between wild and captive animals or the effect of easily accessed food from refuse sites (Warren *et al.,* 2011). This is reflected by the activity budget results which showed that the effect of crop-raiding on the activity budgets of the Gashaka baboons was less than has been found in other studies that have compared the

activities of wild-feeding and food-enhanced baboons (this study, chapter 3 and Warren 2003). It may be that the methods employed here are not precise or sensitive enough to pick up what may be small or inconsistent effects of crop-raiding on the physiological energetic status of the Gashaka baboons.

Perhaps a more likely explanation for this lack of effect is that the benefits of crop-raiding may not be expressed through an improvement in energy balance. It may be assumed that, in order to survive and maintain health, an animal will act to achieve energy balance whether the energy available to it in its habitat is abundant or scarce. The crop-raiding troop may be using their excess energy not to maintain an above average energetic status but instead to invest in other things, such as disease resistance and reproduction. This idea is supported by the fact that Gamgam troop experience shorter inter-birth intervals, lower infant mortality and possibly lower disease related adult mortality than Kwano troop (Higham *et al.*, 2009a).

Rank

In contrast to predictions, low ranking animals exhibited higher UCP levels than both middle and high ranking animals (although the difference was marginally non-significant for middle ranking animals). As detailed in section 4.1.2, previous studies investigating the effect of male rank have detected both negative and positive effects of high rank on UCP levels, with negative effects attributed to the costs associated with male dominance (Emery Thompson *et al.*, 2009; Higham *et al.*, 2011a). However, no study has yet investigated the effect of female rank on UCP values in any non-human primate and since the costs and benefits associated with rank are likely to be very different for males and females, due to fundamental differences in what most often limits their reproductive success (Trivers, 1972), it is difficult to make predictions about

the effect of female rank on UCP levels based on these results. Instead, the effect of female rank on other possible correlates of energy balance can be examined in order to predict the likely effect on UCPs. The positive relationship between female rank and physical condition has been well documented in non-human primates (e.g. Small, 1981; Dittus, 1998; Koenig, 2000; Pusey *et al.,* 2005) although there is limited evidence for a connection in baboons (Silk *et al.,* 2005). A positive relationship, however, has been demonstrated between rank and feeding behaviour in baboons, e.g. higher ranking females experience higher rates of nutrient acquisition and better access to high quality foods than lower ranking females (Barton, 1993; Barton and Whiten, 1993; Altmann and Alberts, 2005), and also with various measures of reproductive success (Bulger and Hamilton, 1987; Altmann *et al.,* 1988; Johnson, 2003; Altmann and Alberts, 2005; Garcia *et al.,* 2006). However, some negative impacts of high rank on reproductive success and health have also been recorded. High ranking female olive baboons at Gombe National Park exhibited higher rates of miscarriage and lower fertility rates than lower ranking females, which was linked to the negative association between factors that promote agonistic competition, such as high androgen levels, and female fertility (Packer *et al.,* 1995). It has also been suggested that parasite loads may be higher amongst more dominant animals due to increased encounter probability, e.g. via more frequent and varied social contact, and this effect has been documented in baboons (Nunn and Altizer, 2006).

Although unexpected, this result might therefore indicate that the higher ranking baboons at Gashaka are experiencing some costs of dominance, such as increased parasitism, which lowers their physiological energetic status relative to lower ranking animals. This hypothesis could be tested by comparing the parasite loads of high and low ranking animals at Gashaka.

Reproductive state

No significant effect of reproductive state on UCP levels was found. Although no previous study has compared the UCP levels of female non-human primates in different reproductive states, this result is not as predicted. Analyses of UCPs in humans have found lowered levels during the early stages of lactation, relative to cycling levels, (analyses from Toba women living in peri-urban communities in Argentina: Ellison and Valeggia, 2003) and the extra energetic costs inherent in pregnancy and, to an even greater degree lactation, were expected to result in lower UCP values for these two groups compared to cycling females. It may be that pregnant and lactating females are successfully off-setting the extra energetic costs with decreased energetic expenditure or increased energetic intake, an idea supported by the calculated energy rate data presented in chapter 3. Both pregnant and lactating animals exhibited significantly lower energy expenditure rates than cycling animals and pregnant animals also exhibited marginally non-significantly higher energy intake rates. However, in chapter three (3.2.2.4) it was suggested that for lactating animals this relatively minor adjustment in energy expenditure rate was unlikely to offset fully the extra costs associated with lactation, which included infant carrying costs as well as milk production costs (Altmann and Samuels, 1992), and it was therefore predicted that lactating animals should demonstrate a physiological cost. This idea is, however, not supported by the UCP results.

4.3.4. Relationship between UCP levels and calculated energy intake and expenditure rates

For Kwano troop only, there was a significant negative relationship between UCP level and median monthly calculated energy expenditure rate. This is as predicted and is consistent with the idea that greater energy expenditure should result in a poorer

physiological energetic status. However, no significant relationship was found between individuals' daily UCP and energy expenditure values for either troop. There are several possible reasons why the relationship with UCP level was significant for median monthly energy expenditure rates but not for the paired, daily values. First, the analysis involving monthly values used a much larger data set including twice as many UCP values and average energy expenditure values drawn from three times as many focal observations. The weaker relationship may therefore just be a result of smaller sample size. Second, the daily calculated energy expenditure rate values are based on between 2.5 and 7.2 hours of in-sight time (mean=5.1±0.2hrs) but the baboons were active for around 12 hours each day, excluding any unobserved nocturnal behaviours. The expenditure data therefore cover, on average, just 42% of the baboons' active period and subsequently may not accurately represent that animal's actual daily energy expenditure. The monthly median values, however, are each based on several days' results and so general shifts in energy expenditure experienced throughout each troop are more likely to be picked up. Third, as discussed in section 4.1.3, UCPs represent an individual's overall energetic status incorporating current energy balance, in terms of recent energy intake and expenditure, and an animal's energy stores, which are dependent on previous as well as current energetic circumstances (Deschner *et al.*, 2008). A monthly average energy expenditure rate measure may therefore provide a better indicator of an individual overall energetic condition than a measurement based on just a few hours from one day.

The lack of relationship between UCP level and calculated energy expenditure rate for Gamgam troop is not as predicted but may be related to the fact that greater energy expenditure is associated with crop-raiding behaviour in this troop (Chapter 3). It may be that on days when the Gamgam troop members expend more energy they also take in

more energy (i.e. extra costs of travelling to and from crop-fields) and therefore maintain a relatively constant physiological energetic status. This idea is supported by the fact that no significant difference was found between the UCP levels of the four urine samples collected on crop-feeding days and all the other Gamgam samples, although this result may also be due to small sample size.

Contrary to predictions, there was little evidence for a consistent, positive relationship between UCP level and calculated energy intake rate and the exact relationship varied according to season, rank, reproductive state and whether daily or mean monthly intake rates were used. The predicted positive relationship between UCP level and intake rate was present for middle ranking animals and, as a trend, for lactating animals when paired, daily UCP and intake rates were examined. In contrast, high ranking and cycling animals exhibited a negative relationship (only a trend for cycling) between paired UCP and intake rates. Similarly, a significant negative relationship between UCP level and mean monthly intake rate occurred during the wet season.

The lack of a consistent relationship between UCP levels and energy intake rates was not as predicted since, it was reasoned, greater energy intake rates would be expected to result in a higher energetic status and positive relationships between food availability, quality or intake have indeed been demonstrated in several studies (table 4.1). However, an argument for the opposite relationship between UCP level and energy intake rate could be made: animals with poorer energetic status may adopt a greater energy intake rate in order to improve their energetic status. These two opposing factors may account for the inconsistent relationship between these variables.

The negative relationship found between UCP level and mean monthly energy intake rate during the wet season may also be related to co-correlations with disease risk and food abundance. Disease risk and food availability both increase in hot and wet conditions (Nunn and Altizer, 2006; Toledo *et al.* 2011), which could lead to high energy intake rates, due to high food availability, alongside low UCP levels, due to high disease prevalence (e.g. Emery Thompson *et al.,* 2009). It may also be the case that animals in poor health increase their energy intake in order to pay for the costs of disease defence.

The apparently weaker effect of energy intake rates on UCP levels compared to the effect of energy expenditure rates, at least for Kwano troop, may indicate that the baboons' physiological energetic status varies more with their energy expenditure than with their energy intake, perhaps due to generally high levels of food availability and quality in their habitat. Interestingly, these results are consistent with the findings of Higham *et al.* (2011a) who showed that UCP levels of male rhesus macaques correlated negatively with behaviours associated with high energy expenditure (time spent travelling, restlessness, copulatory behaviours) but were not related to behaviours associated with energy intake (i.e. feeding time). The macaques in the study by Higham *et al.* (2011a) were provisioned daily and were therefore unlikely to be food limited, unlike the wild primates for which correlations between food availability or quality and UCP level have been demonstrated (Sherry and Ellison *et al.,* 2007; Emery Thompson and Knott, 2008; Emery Thompson *et al.,* 2009; Harris *et al.,* 2009), and the captive primates for which the link between food intake and UCP level has been demonstrated by manipulating food availability (Wolden-Hanson *et al.,* 1993; Deschner *et al.,* 2008; Girard-Buttoz *et al.,* 2011). The lack of relationship between UCP level and food or energy intake in both the Gashaka baboons and the Cayo Santiago macaques (Higham

et al., 2011) may, therefore, be related to a relatively abundant and invariable food supply in their environment.

4.4. Summary of results in relation to the original hypotheses

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

- Gamgam troop did not exhibit significantly higher UCP levels than Kwano troop. The energetic costs of crop-raiding and the possibility that energetic benefits (e.g. elevated calculated energy intake rates) are spent on factors such as disease resistance or reproduction rather than elevated energetic status are discussed.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary

- Contrary to predictions, low ranking animals exhibited the highest UCP levels. Possible energetic costs of dominance are discussed, including increased parasite exposure.
- UCP levels did not vary according to reproductive state. This provides further evidence for the idea that reproductive costs are compensated for by alteration of activity budgets and energy intake and expenditure rates.
- For Kwano troop, UCP levels were negatively related to energy expenditure rate but were unrelated to energy intake rate. The possibility that the baboons' energetic status is more dependent on energy expenditure than energy intake due to Gashaka's generally productive habitat is discussed.

- Little evidence for a consistent relationship between energy intake rate and UCP level was found. Possible explanations, including the co-occurrence of elevated disease risk and food abundance, are discussed.

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability.

- Evidence for seasonal variation in energetic status is presented with higher UCP levels in the wet season than the dry season and significant correlations between UCP levels and rainfall.
- Contrasting relationships between UCP levels and temperature in the wet and dry season are demonstrated. Seasonal variation in the factors affecting energetic status is discussed, including food availability and disease risk.
- Evidence for positive associations between fruit availability and UCP level is presented.

Chapter 5

VARIATION IN GLUCOCORTICOID LEVELS

5.1. Introduction

5.1.1. Glucocorticoids and stress

Stress can be defined as a state in which homeostasis is lost (Reeder and Kramer 2005). Animals respond to stressors by mounting a stress response consisting of physiological and behavioural actions which act to counter the effects of the stressor and restore homeostasis. One of the key physiological responses is the activation of the HPA (hypothalamic-pituitary-adrenal) axis which, among others things, results in the release of glucocorticoid (GC) hormones from the adrenal cortex (figure 5.1).

GCs act to redirect energy away from non-essential functions such as reproduction and growth towards mitigating the immediate effect of the stressor, for example by making energy available for exercising muscles and preparing the body for potential wounds and immune challenges (Sapolsky *et al.*, 2000). Although clearly adaptive, in the short term this process is energetically costly and long term exposure to GCs can have severe negative effects which, in humans, includes hyperglycaemia and insulin resistance (linked to type II diabetes), reduced immune function and growth inhibition (Reeder and Kramer 2005).

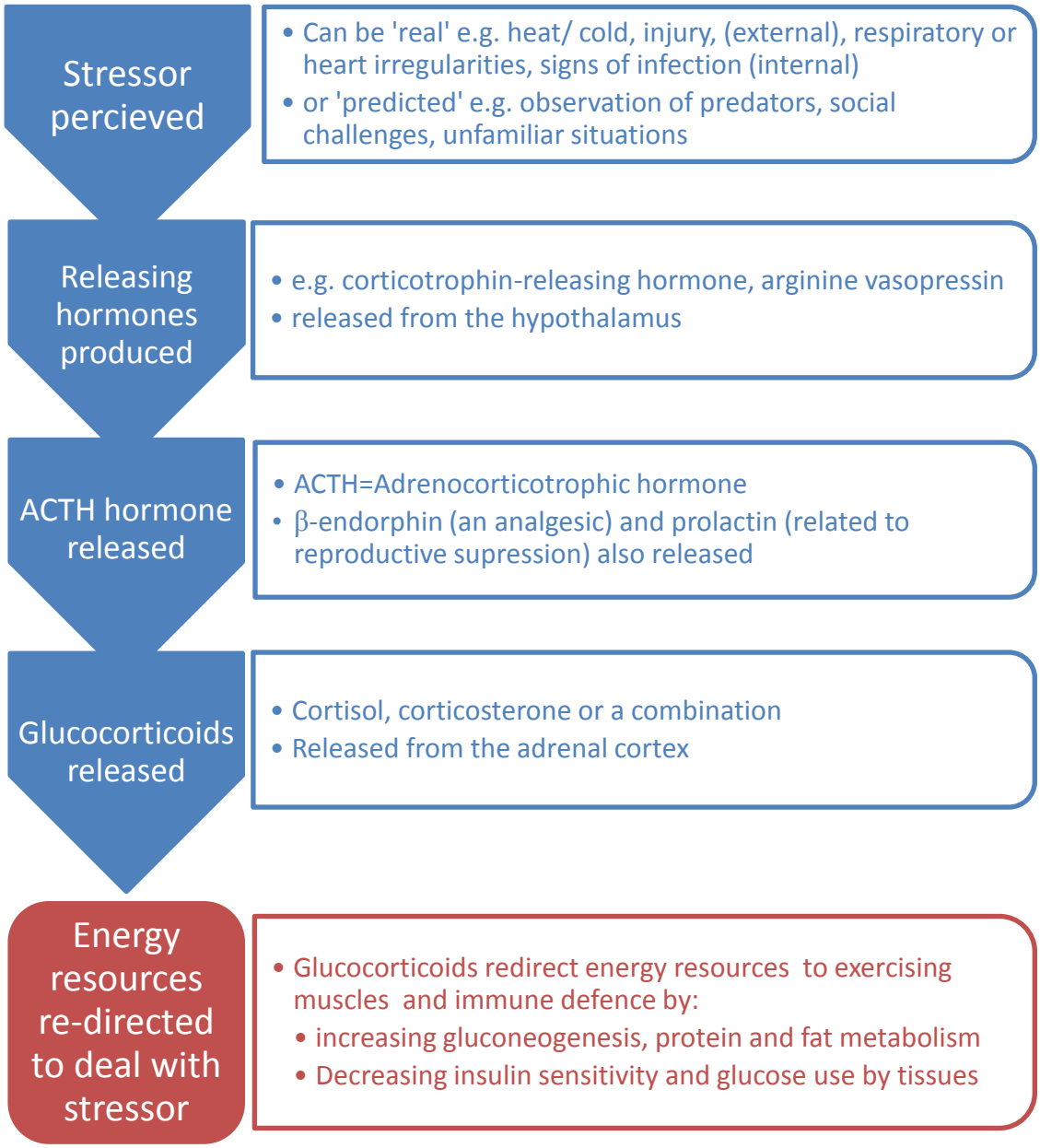

Figure 5.1. Diagram showing overview of HPA axis chain reaction (Herman *et al.*, 2003; Reeder and Kramer, 2005)

The production of GCs is not limited solely to times of exposure to acute stressors. At baseline levels, GCs act to regulate energy acquisition, deposition and mobilisation by influencing feeding behaviour and blood glucose levels (Busch and Hayward, 2009). In many species, baseline GC levels vary predictably in both daily and seasonal cycles, with higher levels associated with times when energy demand is predicted to be greater (Busch and Hayward, 2009; Landys *et al.*, 2006), such as a daily peak when an animal wakes up, in anticipation of the increased levels of energy expenditure associated with

becoming active (Reeder and Kramer, 2005), and seasonal peaks associated with increased external (e.g. severe weather) or internal (e.g. reproduction) energetic demands (Romero, 2002). Baseline GC levels can therefore be used to examine the energetic demands that certain environmental or life-history variables place on an animal (Emery Thompson *et al.*, 2010).

GCs have been used frequently as a means to assess the physiological costs of social interactions and variations in essential resources in non-human primates and other vertebrates (e.g., van Schaik *et al*., 1991; Creel, 2001, 2005; Goymann *et al*., 2001; Muller and Wrangham, 2004; Beehner *et al.*, 2005) and have also been used as an indicator of individual fitness and population health (Chapman *et al.*, 2007 but see Romero 2004 for problems with this approach). Measurement of baseline GC values can provide a measure of fitness for three reasons (Bonier *et al.* 2009): 1) elevated GCs divert resources from reproduction and long-term survival to dealing with the immediate stressor and therefore potentially decrease fitness; 2) lower quality individuals may find an environment more challenging than higher quality individuals and therefore have higher baseline GC levels alongside lower overall fitness; 3) there are direct fitness consequences of chronically high GC levels on metabolic, vascular, immune, growth and reproductive function (Wingfield and Sapolsky, 2003). However, the actual relationship between baseline GCs and various proxies and measures of fitness is not always easy to predict and can vary according to environmental factors, previous exposure to stressors (both recent and during development), and individual life-history stage, reproductive state and reproductive strategy (Bonier *et al.* 2009).

The measurement of GCs in faecal samples provides a non-invasive method for quantifying the stress levels in wild animals (Whitten *et al.*, 1998; Hodges and

Heistermann, 2003). Measures from faecal samples represent average GC levels over a period of a few hours to a few days and thus provide more accurate assessment of long-term GC levels than would be obtained from a single blood sample (Millspaugh and Washburn, 2004). In this way GC levels from faecal samples may represent both an individual's basal GC levels, indicating their current energetic response to environmental or life-history conditions, as well as the results of any acute stressor experienced by the animal within the previous few days. Although distinguishing between the effects of basal and stress induced variation in GC levels in this situation is practically impossible, in either case, high levels indicate negative experiences, be they chronic or acute.

5.1.2. Glucocorticoids in relation to factors of interest in this study

The following sections summarise the previous research on non-human primates and other vertebrates relating to the influence on GC levels of the factors of interest in this study namely: the effect of season and weather; food availability and enhancement; energetic status; activity budgets; reproductive state and rank.

Relationship with season/weather

Seasonal cycling in baseline GC levels has been observed in the majority of vertebrate species that have been studied (Romero, 2002). Elevated GC levels have been associated with harsh seasons (e.g. winter (red deer: Huber *et al.*, 2003; mule deer: Saltz and White, 1991) dry season (ring-tailed lemur: Cavigelli, 1999; tufted capuchin: Lynch *et al.*, 2002; pampas deer: Pereira *et al.*, 2006; white-faced capuchin: Carnegie *et al.*, 2011) or with energetically costly life-history stages (e.g. breeding season: review by Romero, 2000; prior to hibernation e.g. Armitage, 1991; Reeder *et al.*, 2004). However, all of the species mentioned in these studies are seasonal breeders, a fact which means

that it is difficult to disentangle the effects of internal (reproductive) and external (climatic) variables.

Several recent studies of non-seasonal breeding species have recorded significant effects of season or weather on GC levels. Female yellow baboons in the arid Amboseli basin, in Kenya, had higher GC levels during the dry season than during the wet season and during months with higher temperatures and lower rainfall (Gesquiere *et al.* 2008). Similarly, elevated GC levels were found in male olive baboons during a severe drought (Sapolsky, 1986). Chacma baboons in South Africa experienced elevated GC levels during the winter months when daylight hours and temperatures are at their lowest (Weingrill *et al.,* 2004), although no seasonal effect was detected for the Botswanan chacma baboons, which experience less extreme seasons (Crockford *et al.,* 2008). In geladas living in the Simien Mountains of Northern Ethiopia, elevated GC levels were associated with low temperatures and high altitude (Beehner and McCann, 2008).

The relationship between climate and GC levels is explained partly by the fact that GC release is stimulated by temperatures outside an animal's thermal neutral zone. GCs then help to activate mechanisms such as shivering, if temperatures are too low, and sweating or panting, if temperatures are too high in order to maintain a safe body temperature (Randall *et al.,* 2001; Carnegie *et al.,* 2011). Alternatively, weather variables could influence GC levels via their effect on food availability and quality (see below) or via their effect on other factors such as disease risk, which increases during the wet season in tropical regions (Freeland, 1976; Nunn *et al.,* 2006). Associations between disease and elevated cortisol levels have been found in free-ranging primates. For example, in Ugandan red colobus monkeys, parasite infection was found to be associated with elevated cortisol levels (Chapman *et al.,* 2007) and in male chimpanzees

GC levels and intestinal parasite richness were directly, and positively, related (Muehlenbein and Watts, 2010). These results suggest that environmental stressors are associated with increased GC levels in non-seasonal breeders. At Gashaka the most likely environmental stressors are high rainfall and high temperatures since these are higher than at most other baboon habitats.

Relationship with food availability and intake

Several non-human primate studies have demonstrated a negative relationship between food availability or intake and GC levels. In chimpanzees, elevated GC levels were associated with periods of low fruit consumption in male animals (Muller and Wrangham, 2004) and in lactating females (Emery Thompson *et al.,* 2010). Female ring-tailed lemurs with low food intake rates experienced elevated GC levels (Pride, 2005). In Sykes' monkeys, elevated GC levels were associated with both low availability of a preferred food item and high availability of a low quality, hard to process food item (Foerster and Monfort, 2010). Elevated GC levels have also been associated with poor quality diets in red colobus monkeys (Chapman *et al.*, 2007) and low fruit availability in howler monkeys (Behie *et al.,* 2010). Negative associations between food availability and GC levels have been recorded in several other vertebrate species (mule deer: Saltz and White, 1991; kittiwakes: Kitaysky *et al*., 1999; African elephants: Foley *et al*., 2001; song sparrows: Clinchy *et al*., 2004; grizzly and black bears: Wasser *et al*., 2004; albatross: Angelier *et al.,* 2007a; Florida scrub-jay: Schoech *et al.,* 2007; barn swallows: Jenni-Eiermann *et al*., 2008; elephant seals: Ortiz *et al*., 2001).

At a proximate level, the relationship between food intake and GCs is controlled by the fact that GCs are positively related to appetite; elevated baseline GCs increase appetite

and their daily peak tends to correspond with a peak in an animal's appetite (Sapolsky, 2000). However, the immediate effect of acute stressors is actually to decrease appetite, due to the first wave of stress hormones, released prior to GCs. Another complication is that high levels of GCs stimulate insulin secretion, which suppresses appetite (discussed further below) and means that stress-induced GC levels are associated with decreased appetite (Sapolsky, 2000). These factors help account for the association between increased stress, and GC levels, and reduced feeding behaviour and weight loss observed in several laboratory stress studies (Rybkin *et al.,* 1997; Harris *et al.,* 1998; Tamashiro *et al.,* 2006; Tamashiro *et al.,* 2007).

Little attention has been paid to the effect of food-enhancement on the GC levels of free-ranging primates. It seems likely that food-enhancement will have a similar effect to increases in abundance of wild-foods and therefore result in lowered GC levels. In free-ranging Sykes' monkeys, increases in provisioning rate were associated with reduced GC levels, but only when alternative high quality wild-foods were not available (Foerster and Monfort, 2010). It has also been suggested that the GC levels of food-enhanced animals (like captive animals) will be more closely related to psychosocial stress than energetic stress, whereas the opposite will be true for entirely wild-feeding animals, due to their lower and more variable energetic status (Muller and Wrangham, 2004). The relationship between GC levels and environmental variables, wild-food availability or energy expenditure may therefore be weaker for food-enhanced groups than for entirely wild-feeding ones. However, certain types of food-enhancement, such as crop-raiding, which involve risks may act as stressors and initiate an acute stress response in the raiding animals. This effect has been observed in elephants, where crop-raiding animals had elevated faecal GC levels relative to non-crop-raiding animals (Ahlering *et al.*, 2011).

Relationship with energy expenditure and activity budgets

There is a fundamental link between GCs and energy expenditure since GCs are instrumental in the mobilisation of energy reserves during physical activity (Tharp, 1975; Girard and Garland, 2002). Early laboratory experiments found that injected GCs increased the amount of work an animal, or isolated muscle, is able to do before becoming exhausted. They also found that high levels of exercise lead to progressive increases in GCs whereas low levels of exercise could either increase, decrease or have no effect on GC levels (Tharp, 1975). Laboratory studies have also found relationships between GC levels and voluntary locomotion, for example, a study of voluntary wheel-running in mice found that plasma GC levels were directly related to the number of wheel revolutions in the 20 minutes before blood sampling (Girard and Garland, 2002).

Several studies of birds also provide evidence for the link between GC levels and increased locomotory behaviour. For example, experimentally elevated GC levels were associated with more time spent flying and foraging by free-ranging kittiwakes (Angelier *et al*., 2007b) and GC levels were positively correlated with distance travelled during foraging trips in the wandering albatross (Angelier *et al.*, 2007a).

Only a few studies have investigated the relationship between GC levels and energy expenditure or activity budgets in free-ranging primates. In ring-tailed lemurs, GC levels correlated positively with 'feeding effort', a ratio of time spent travelling and 'actively' foraging to time spent sleeping, resting and 'passively' foraging (Cavigelli, 1999). In female long-tailed macaques, urinary GC levels were positively associated with time spent foraging in the two hours prior to sample collection but not related to time spent travelling (van Schaik *et al.*, 1991). In South African chacma baboons GC

levels were higher in months when less time was spent resting (Weingrill *et al.,* 2004). In addition, although the effect of time spent travelling on GC levels was not directly tested, months with more travel time were associated with longer daylight hours and lower GC levels (Weingrill *et al.*, 2004). In contrast, a study of Botswanan chacma baboons found no effect of activity budget variation on GC levels (Crockford *et al.* 2008). Time spent feeding did not significantly predict GC levels in yellow baboons, at Amboseli. However, during the dry season, when GC levels were elevated, the baboons did spend more time feeding and less time resting compared to the wet season (Gesquiere *et al.*, 2008).

Relationship with insulin and C-peptides

As discussed above, GC levels are positively related to energy expenditure and negatively related to energy intake and balance. As indicators of physiological energy balance, insulin and UCP levels are therefore expected to correlate negatively with GC levels and this effect has been demonstrated. For example, a study of normal- and low-weight anorexia nervosa patients found that GC levels correlated negatively with BMI, insulin and both plasma and urinary C-peptide levels (Mocanu *et al.*, 2003). In addition to this, rats kept in a socially stressful environment showed low insulin levels, relative to controls, alongside elevated GC levels (Tamashiro *et al.,* 2006).

However, due to the central role GCs and insulin play in controlling energy metabolism the physiological relationship between these two hormones is complex (Hardy, 1981). The major roles of insulin and GCs tend to have opposing effects. Insulin acts to decrease blood glucose levels, glucose production, fat breakdown and appetite while increasing protein production and fat deposition. In contrast, GCs increase blood glucose levels, glucose production, fat breakdown and appetite and decrease protein

production and fat deposition (Sapolsky *et al.,* 2000). GCs also reduce the uptake and utilisation of insulin in fat and muscle cells (Dallman *et al.* 1993). However, in other areas the effects of insulin and GCs are synergistic, e.g. in lipogenesis and hepatic glycogen deposition (Sapolsky *et al.*, 2000).

Increases in GC levels can cause increases in insulin levels and vice versa (Dalman *et al.,* 1993; Sapolsky *et al.,* 2000). If blood glucose levels are high this stimulates the secretion of insulin, which will cause glucose levels to fall. These low blood glucose levels will, in turn, stimulate GC release which, via increasing appetite and glucogenesis, will act to raise blood glucose levels again, which will then, once again, stimulate insulin secretion (Hardy, 1981). The relationship between GCs and insulin has been demonstrated in multiple sclerosis patients, where treatment with corticosteroids increased plasma insulin and C-peptide levels (Laznic *et al.,* 2011), and in adrenalectomised rats receiving corticosterone replacement treatment, where plasma insulin levels increased in step with corticosterone level (Dallman *et al.,* 2007). Another study demonstrated elevated insulin levels alongside elevated GC levels in rats maintained on a high-fat diet (Tamashiro, *et al.* 2006).

Effect of reproductive state

Elevated GC levels during pregnancy, which are related to the production of placental corticotrophin-releasing hormone (Carnegie *et al.,* 2011), have been demonstrated many times in primates (e.g. yellow baboon: Gesquire *et al.,* 2008; mandrill: Setchell *et al.,* 2008; French *et al.,* 2004; rhesus macaque: Hoffman *et al.,* 2010; black tufted-ear marmoset: Smith and French, 1997; cotton-top tamarin: Ziegler *et al.,* 1995; white-faced capuchin: Carnegie *et al.* 2011; ring-tailed lemurs: Cavigelli, 1999; humans: Lockwood *et al.,* 1996; McLean and Smith, 1999), with the most substantial elevations

towards the end of pregnancy (Zakar and Mitchel, 1998; Carnegie *et al.* 2011). However, not all studies have demonstrated this effect (Sykes monkey: Foester and Monfort, 2010) and it has been suggested that environmental stressors could override the physiological effects of pregnancy on GC levels (Foerster and Monfort, 2010).

Elevated GC levels, relative to reproductive cycling levels, have also be found in lactating non-human primates, apparently due to the high energetic costs of lactation and/or increased exposure or vulnerability to stress (chacma baboons: Weingrill *et al.*, 2004; chimpanzees: Emery Thompson *et al.*, 2010; rhesus macaque: Hoffman *et al.*, 2010). This pattern has also been seen in carnivores (spotted hyenas: Goymann *et al*., 2001) and rodents (Cascade golden-mantled ground squirrel: Boswell *et al*., 1994; degu: Kenagy *et al*., 1999; yellow-pine chipmunks: Kenagy and Place, 2000). However, other non-human primate studies have found no difference between cycling and lactating GC levels (e.g. Chacma baboon: Engh *et al.*, 2006; Mandrill: Setchell *et al.*, 2008).

Effect of female rank

The relationship between dominance rank and stress in social animals has been a major focus of GC research (Creel, 2001). The original assumption was that low ranking animals will have higher GC levels due to a lack of control and higher degree of uncertainty experienced by these animals (Sapolsky, 1992). However, this pattern is, in reality, uncommon in studies of wild animals and the actual relationship between rank and GC levels seems to vary according to the stability of the hierarchy and the energetic costs associated with dominance (Creel, 2001; Abbott *et al.*, 2003; Muller and Wrangham, 2004).

Previous studies investigating the relationship between GCs and rank in female non-human primates have found mixed results. Many studies have found no effect of rank on GC excretion (van Schaik *et al.,* 1991; Hoffman *et al.,* 2010; Setchell *et al.,* 2008; Weingrill *et al.,* 2004; Crockford *et al.,* 2008) while others have found the highest levels in high ranking animals (Cavigelli 1999; Cavigelli *et al.,* 2003), low ranking animals (Maestripieri *et al.* 2009; Emery Thompson *et al.,* 2010; Foerster and Monfort 2010) or even middle ranking animals (Stavisky *et al.,* 1997, referenced in Whitten *et al.,* 1998). Meta-analyses of the primate and vertebrate literature conclude that the relationship between GC levels and rank is determined by the relative costs to dominant and subordinate animals of how rank is achieved, maintained and how resource access is controlled (Abbott *et al.,* 2003; Goymann and Wingfield, 2004)

5.1.3. Predictions

In this chapter, data on the GC levels of the Gashaka baboons are presented. Following on from the analyses in chapters 3 and 4, the effects of troop, season, rank and reproductive state on GC level are assessed, as are the relationships between GC level and weather and fruit index variables, calculated energetic measures (intake and expenditure rate) and UCP values. The following predictions were developed from the three study hypotheses:

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

Prediction: Gamgam troop will have lower GC levels than Kwano troop because it is less energetically stressed.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary.

Predictions:

a. Pregnant females will have higher GC levels than cycling females due to physiological processes inherent to pregnancy.
b. Lactating females will have higher GC levels than cycling females due to greater energetic stress and exposure to stressors.
c. GC levels will be higher for lower ranking animals.
d. GCs will correlate negatively with calculated energy intake rate
e. GCs with correlate positively with calculated energy expenditure rate
f. GCs will correlate negatively with urinary C-peptide levels

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability.

Predictions:

a. GCs will correlate negatively with the fruit indices, due to reduced energetic stressors when more food is available.
b. GCs will correlate positively with rainfall and temperature since heavy rainfall and high temperatures increase disease risk.

5.2. Results

5.2.1. Overview of GC data and methodological analyses

For methods of faecal sample collection and analysis for GC content see chapter 2, sections 2.2.2 and 2.3.1, respectively. In order to account for the time it takes for hormones circulating in the blood to be excreted as metabolites in faeces, a standard excretion lag of 2 days was used (Wasser *et al.*, 1994; Heistermann *et al.*, 1996; Higham

et al., 2009b). This means that GC values were matched up with other data (e.g. weather, UCP and focal observation data) collected two days prior to the collection of the faecal sample. The analyses were also all performed using an excretion lag of one day but this did not affect any of the relationships reported here.

A total of 211 GC values are used in the following analyses. The data range from 160 to 8220 ng of GC per g dry faecal mass and are right skewed. Table 5.1 gives the number of samples and mean GC values for each individual and the two troops.

Table 5.1. Median and range of GC concentrations for individual focal animals and troops

FocalID	Median GC concentration[1] (range in brackets)	n
BRA	2011 (3394)	11
DRK	2500 (2098)	7
FDI	1118 (996)	8
KRM	1274 (2109)	8
KYE	3840 (6164)	6
LDI	1516 (1962)	9
LMI	2178 (4104)	12
MOM	2004 (1949)	11
SDY	1864 (3291)	13
TOJ	2679 (3259)	11
YMK	1966 (2860)	12
Kwano[2]	**1949 (7028)**	**108**
BUD	1007 (1322)	17
KNE	860 (3492)	24
MMK	917 (7900)	25
MMW	875 (2381)	11
STR	1229 (4542)	26
Gamgam[2]	**971 (8060)**	**103**
Both troops	**1383 (8060)**	**211**

1. GC concentration in ng/g dry faecal mass
2. Troop values are medians from all GC values for that troop

Effect of collection time on GC levels

There was no significant difference between the GC levels of samples collected during different hours of the day (Kruskal Wallis tests: χ^2=16.606, d.f.=10, p=0.084, figure 5.2)

or between samples collected in the morning (between 06:00 and 12:00) and in the afternoon (between 12:01 and 18:00) (Mann Whitney U test: U=4655, p=0.102).

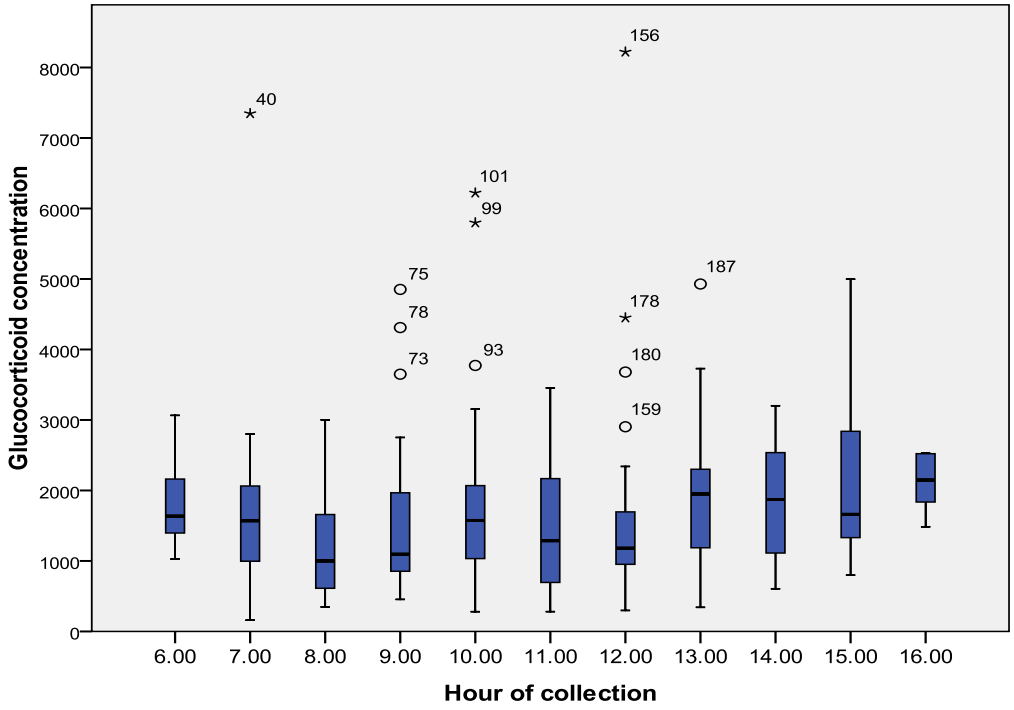

Figure 5.2. Box-plot showing GC levels (ng/g dry faecal mass) of samples collected at different hours of the day.

Effect of faecal sample mass on GC concentration

Extremes of faecal sample size may affect the measurement of GCs in a sample. For example, bird faecal samples with a wet mass of less than 0.02g were found to result in proportionately higher GC levels (Millspaugh and Washburn, 2004). The samples collected for the present study varied between 0.07 and 1.48 g dry mass but there was no significant effect of sample mass on GC level (Spearman's rank correlation: r_s=-0.075, n=224 (includes replicate samples), p=0.262, figure 5.3). This result suggests that the faecal samples collected for the present study fell within a mass range that did not affect measurement of GC metabolites.

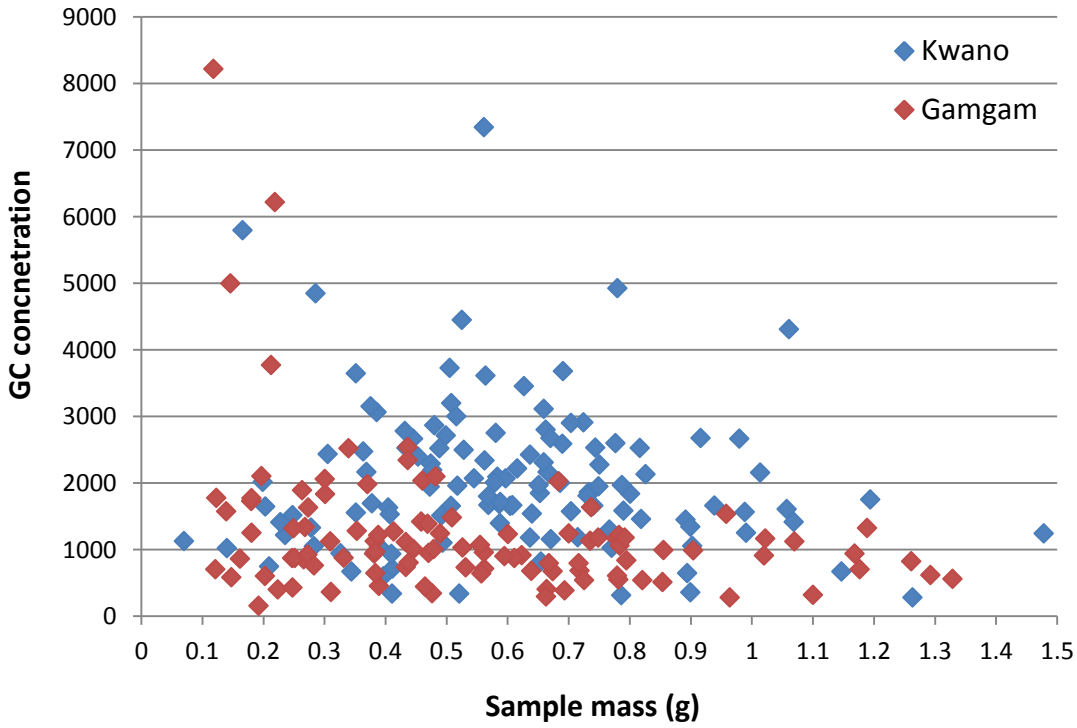

Figure 5.3. Scatter plot showing the variation in GC concentration (ng/g dry faeces) with faecal sample mass for both troops

5.2.2. Variation in GC level between different troops, seasons, ranks and reproductive states

The effect of the categorical variables, troop, season, rank and reproductive state, on GC level were assessed by building GLMMs with Log_{10} transformed GC data (n=211) as the dependent variable and with faecal sample number and ID fitted as random effects. The categorical variables troop, season, rank and reproductive state were then added to the GC 2-factor null model (which contained only the two random factors) in turn as fixed effects. All 2-way interactions between the categorical variables were also investigated. Full results of statistical analyses relating to all the models in this chapter are presented in appendix 6c.

The addition of troop significantly improved the fit of the GC 2-factor null model (D=14.03, d.f.=1, p<0.001), with Kwano troop members exhibiting significantly higher GC levels than Gamgam troop members (z=5.27, p<0.001, figure 5.4).

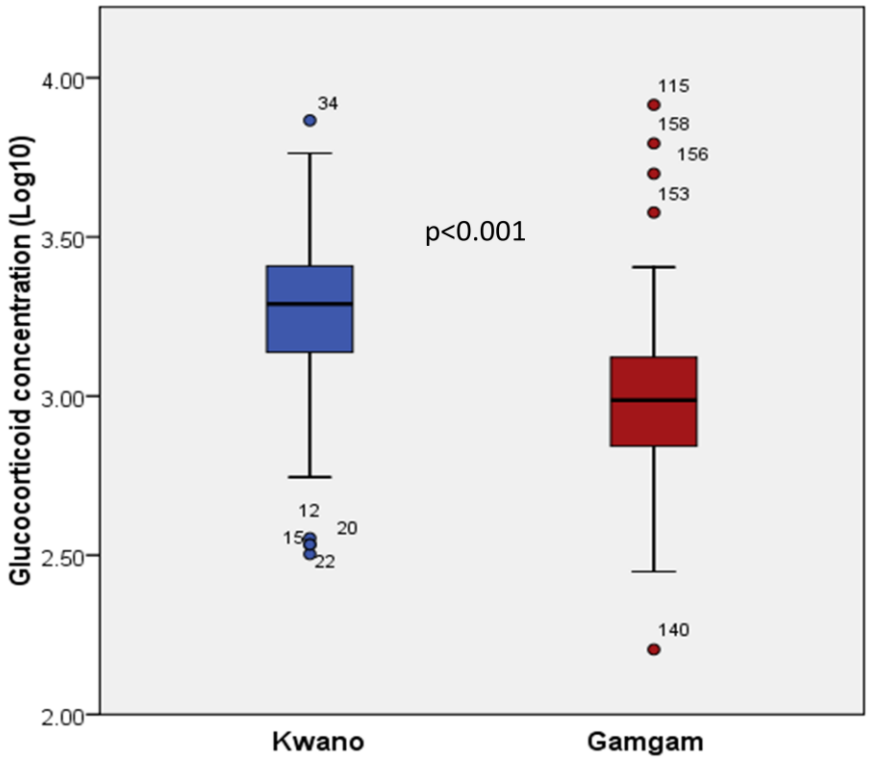

Figure 5.4. Box-plot showing GC levels (ng/g dry faeces) for focal animals belonging to different troops.

The addition of reproductive state did not significantly improve the fit of the GC 2-factor null model (D=0.09, d.f.=2, p=0.956). However, when values from pregnant females were split into those collected during the first (P1), second (P2) and third (P3) trimesters, to create a pregnancy stage variable the fit of the null model was significantly improved (D=11.85, d.f.=3, p=0.008). The GC levels of samples from P3 animals were significantly higher than those from P1 (z=3.08, p=0.002), P2 (z=3.07, p=0.002) and non-pregnant (z=2.86, p=0.004) animals but there were no significant differences between any of the other categories (figure 5.5, appendix 6c, table A6.ix)

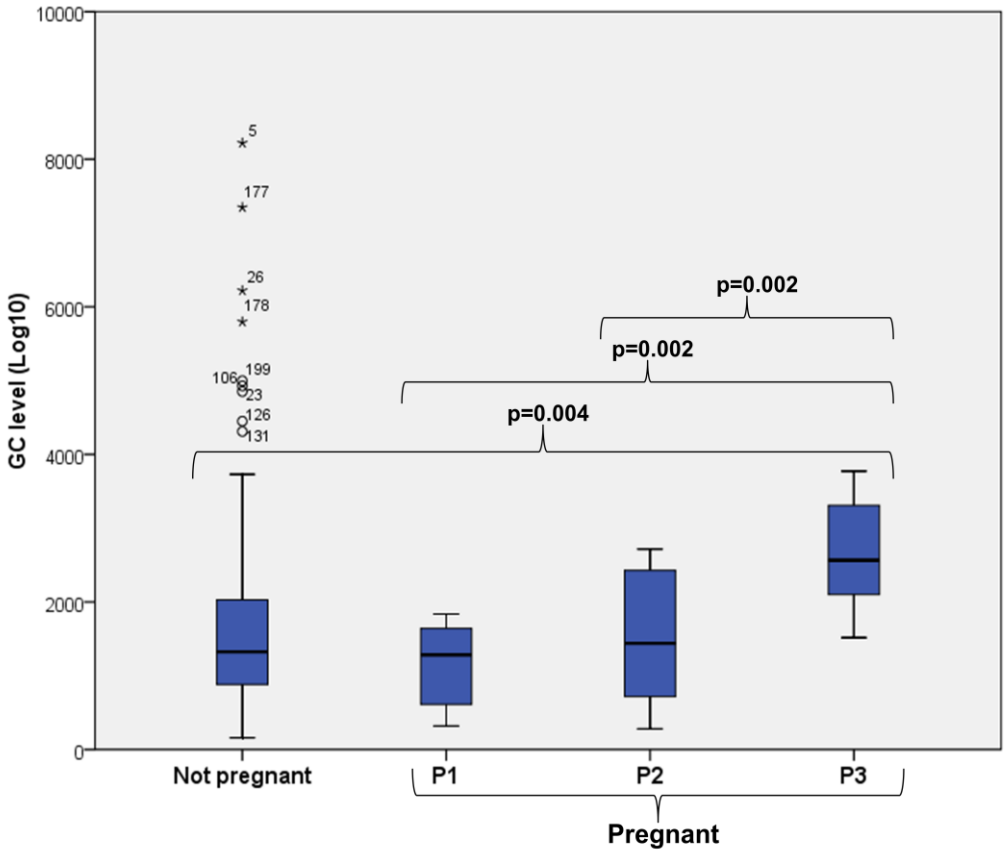

Figure 5.5. Box-plot showing the effect of pregnancy stage on the GC levels (ng/g dry faeces) of focal animals.

The fit of the GC 2-factor null model was not significantly improved by the addition of either season or rank and there were no significant interaction effects between the categorical variables (appendix 6c, table A6ix).

5.2.3. Comparison of GCs with weather and fruit index variables

As in chapters 3 and 4, each of the weather and fruit index variables was added to the GC 2-factor null model, in turn, as fixed effects. The effects of each of the weather and fruit index variables on GC level were considered separately for each troop due to the substantial effect troop has on GC level (figure 5.4).

30-day rainfall was significantly and positively related to GC from both troops (troop interaction effect: D=0.17, d.f.=1, p=0.680; Kwano relationship: z=2.82, p=0.005; Gamgam relationship: z=2.48, p=0.013; figure 5.6).

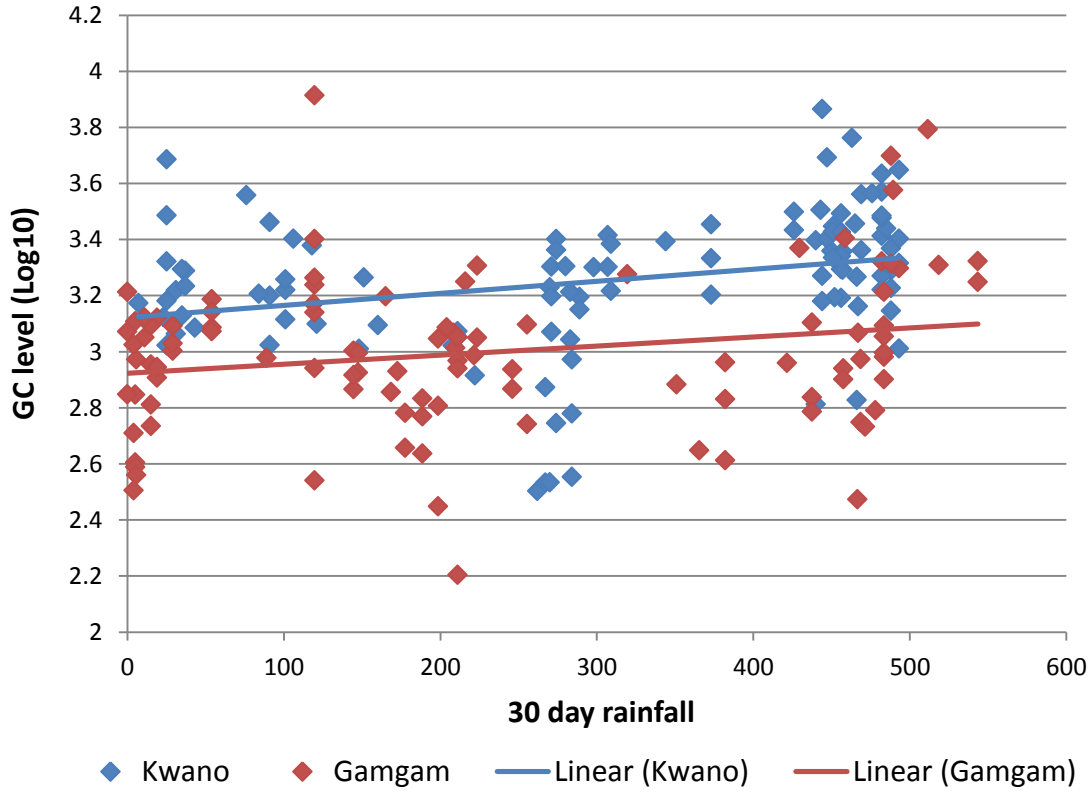

Figure 5.6. Scatter plot showing the effect of troop on the relationship between GC levels (ng/g dry faeces) and 30-day rainfall (mm). The lines represent the average linear relationship between the two variables for all individuals from each troop, as predicted by the GLMM.

Total- and vine-fruit index were negatively related to GC level. These relationships were highly significant for Kwano troop (total: z=4.89, p<0.001; vine: z=5.66, p<0.001) but only present as a trend for Gamgam troop (total: z=1.68, p=0.093; vine: z=1.91, p=0.057, figure 5.7).

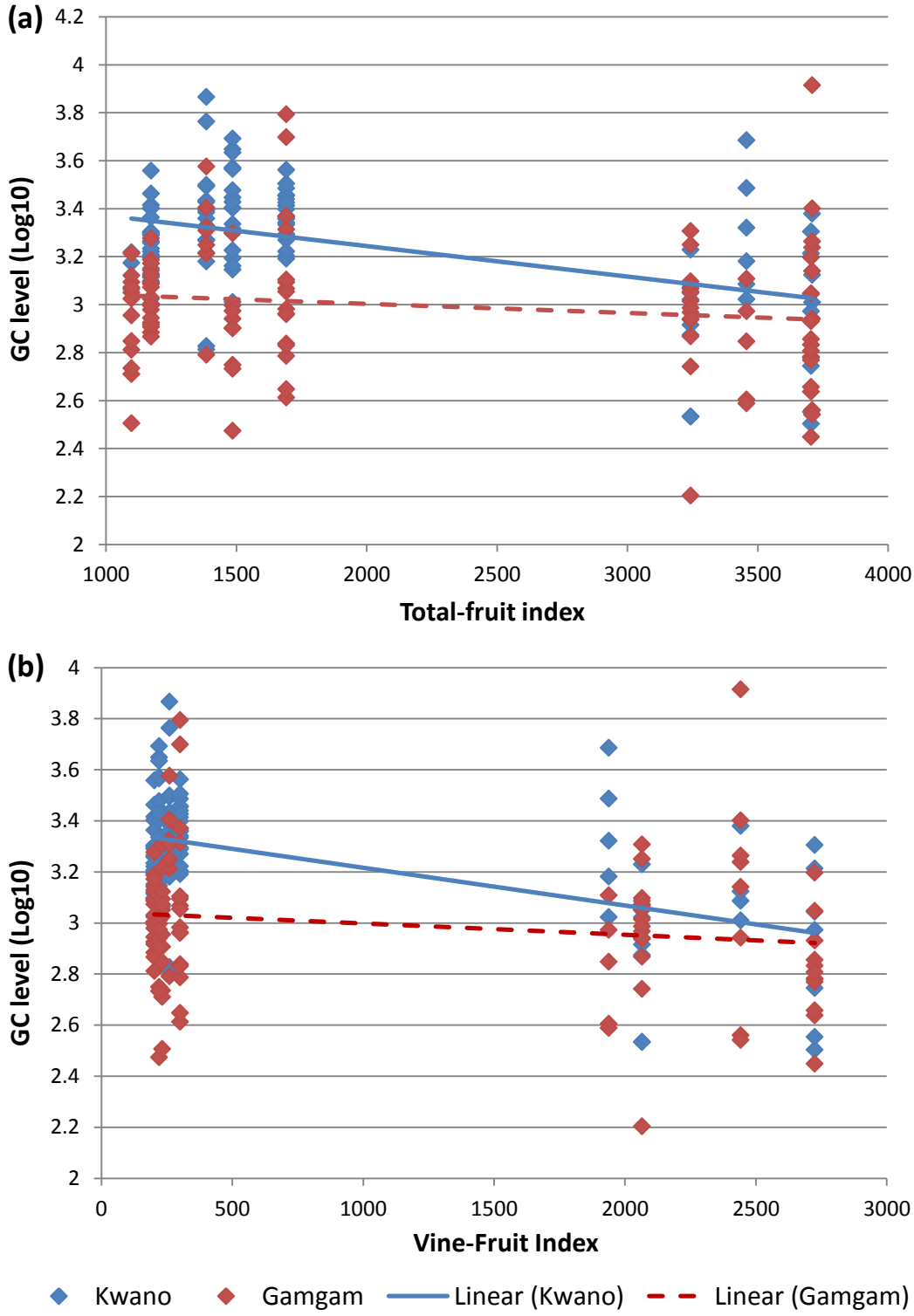

Figure 5.7. Scatter plot showing the effect of troop on the relationship between GC levels (ng/g dry faeces) and both (a) total-fruit index and (b) vine-fruit index. The lines represent the average linear relationship between the two variables for all individuals from each troop, as predicted by the GLMM.

Tree-fruit index was significantly, positively related to GC level for Kwano troop (z=2.90, p=0.004) but un-related for Gamgam troop (z=0.93, p=0.352) (figure 5.8).

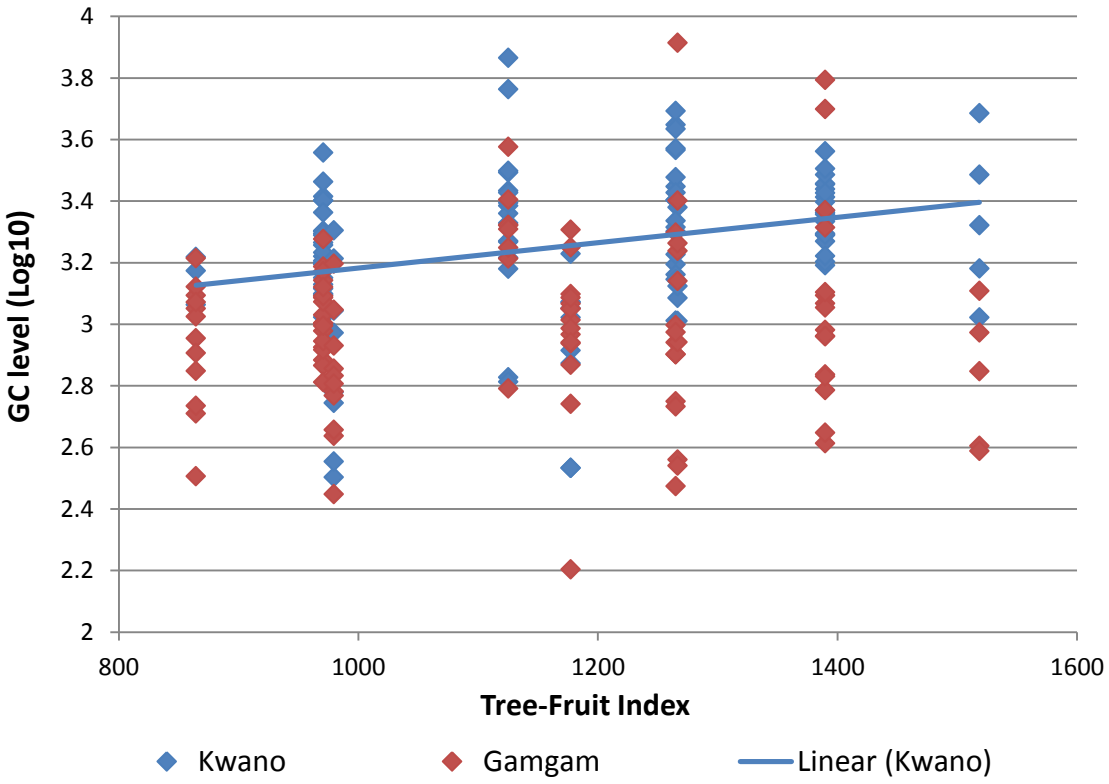

Figure 5.8. Scatter plot showing the effect of troop on the relationship between GC levels (ng/g dry faeces) and tree-fruit index. The line represents the average linear relationship between the two variables for all individuals from Kwano troop, as predicted by the GLMM.

None of the temperature variables or daily rainfall were significantly related to GC level for either troop (appendix 6c, table A6.ix).

<u>Interactions with season, rank and reproductive state</u>

The addition of the season interaction effect significantly improved the fit of the GC models containing 30-day rainfall and the three fruit indices (plus troop and all 2-way interactions) (30-day rainfall: $D=17.97$, $p<0.001$; total-fruit index: $D=13.53$, $p=0.004$; tree-fruit index: $D=15.27$, $p=0.002$; vine-fruit index: $D=9.52$, $p=0.023$; d.f.=1 in all cases). Season did not interact significantly with any of the temperature variables or daily rainfall (appendix 6c, table A6.ix).

For Kwano troop the relationship between GC level and 30-day rainfall was significant and positive during both the dry (z=2.11, p=0.035) and wet (z=5.20, p<0.001) seasons but for Gamgam troop this relationship was only significant during the wet season (z=3.28, p=0.001) (Figure 5.9).

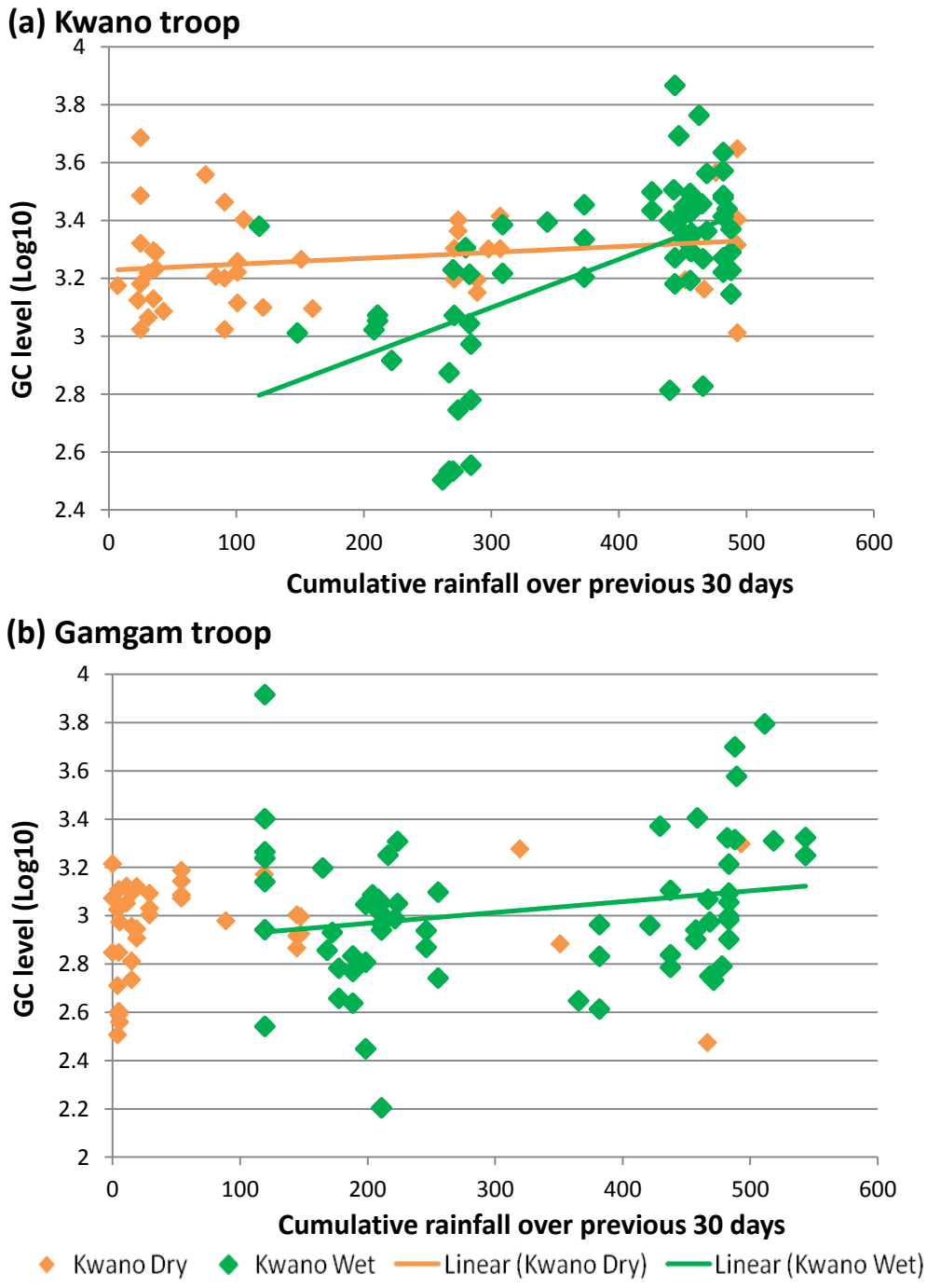

Figure 5.9. Scatter plots showing the effect of season on the relationships between GC levels (ng/g dry faeces) and 30-day rainfall (mm) for (a) Kwano troop and (b) Gamgam troop. The lines represent the average linear relationship between the two variables for all individuals from each troop during each season, as predicted by the GLMM.

The negative relationships between GC level and both total- and vine-fruit index were significant during both the dry (total: $z=1.96$, $p=0.050$; vine: $z=2.23$, $p=0.026$) and wet (total: $z=5.66$, $p<0.001$; $z=6.04$, $p<0.001$) seasons for Kwano troop (figures 5.10a and b) but only during the wet season for Gamgam troop (total: $z=3.56$, $p<0.001$; vine: $z=3.20$, $p=0.001$) (figures 5.10c and d).

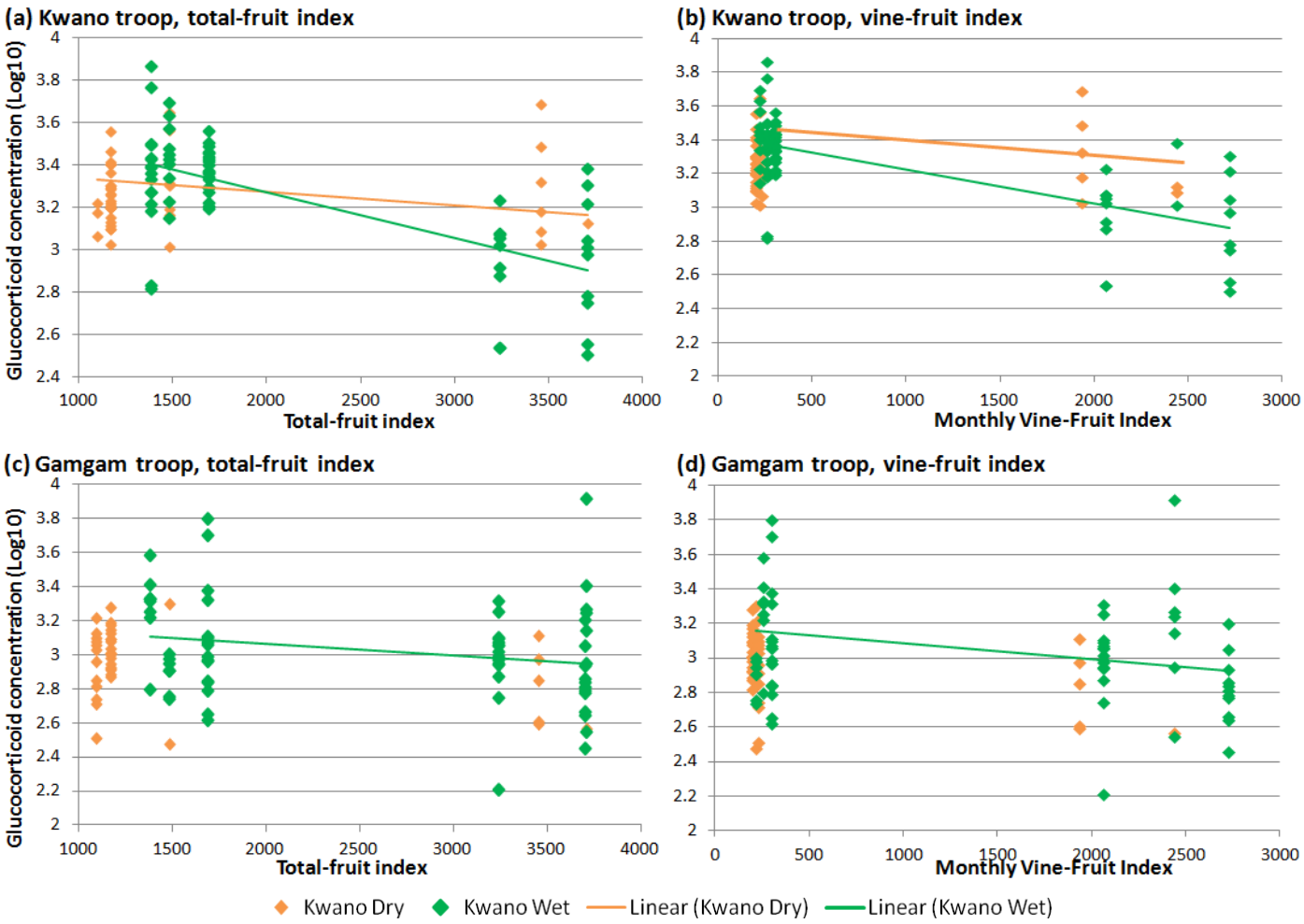

Figure 5.10. Scatter plots showing the effect of season on the relationships between GC levels (ng/g dry faeces) and both total- and vine-fruit index for both troops. The lines represent the average linear relationship between the two variables for all individuals from each troop during each season, as predicted by the GLMMs.

During the wet season the relationship between GC level and tree-fruit index was significant and positive for both Kwano ($z=4.85$, $p<0.001$) and Gamgam ($z=2.46$, $p=0.014$) troop (figure 5.11) but was not significant for either troop during the dry season (appendix 6c, table A6.ix).

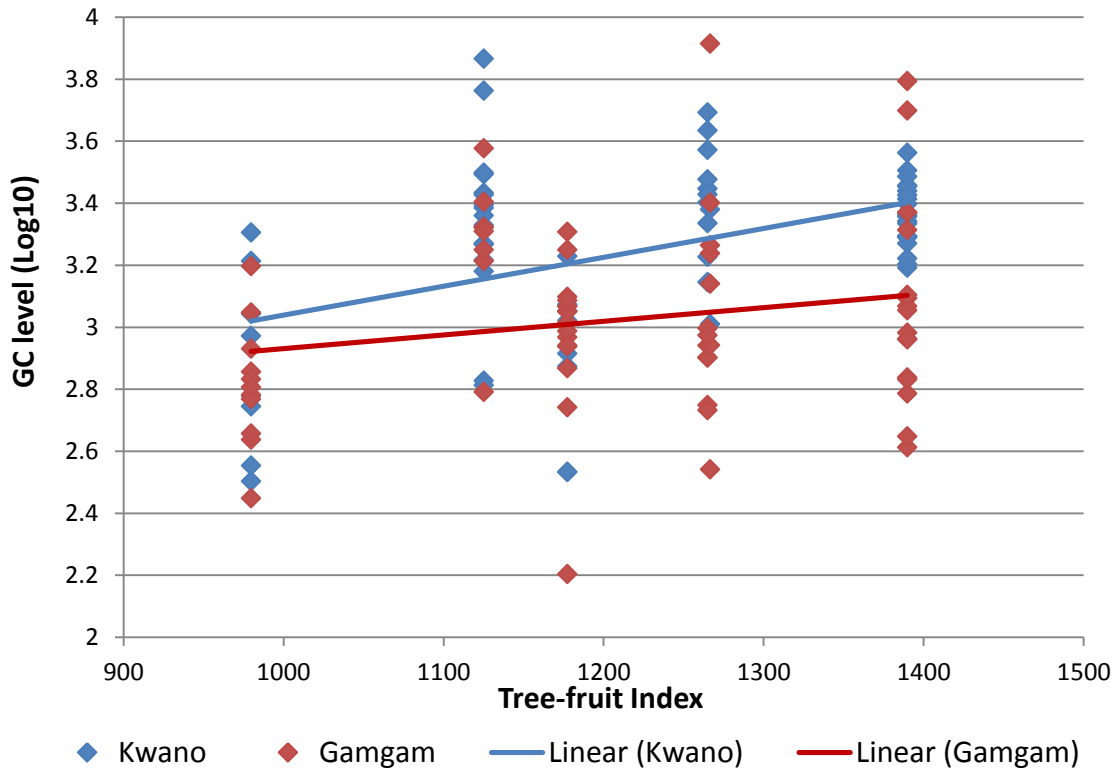

Figure 5.11. Scatter plot showing the effect of troop on the relationship between GC levels (ng/g dry faeces) and tree-fruit index during the wet season. The lines represent the average linear relationship between the two variables for all individuals from each troop, as predicted by the GLMM.

Rank did not interact significantly with any of the weather and fruit index variables for either troop (appendix 6c, table A6.ix).

The addition of the reproductive state interaction effect significantly improved the fit of the GC models containing troop and total-fruit index ($D=25.81$, $d.f.=1$, $p<0.001$) or

vine-fruit index (D=22.55, d.f.=1, p=0.001) but not of the models containing troop and tree-fruit index or any of the weather variables (appendix 6c, table A6.ix).

In both troops the negative relationships between GC level and both total- and vine-fruit index were significant for pregnant and lactating animals but not for cycling animals (table 5.2, figure 5.12).

Table 5.2. Results of z tests from GLMMs showing the relationship between GC level and either total- or vine-fruit index for animals in each reproductive state from each troop. Table shows the results of two models, each containing the categorical variables troop and reproductive state and one of the following: total-fruit index or vine-fruit index, as fixed variables.

	Kwano troop				**Gamgam troop**			
Reproductive state	**Coefficient**	**s.e.**	**z**	**p**	**Coefficient**	**s.e.**	**z**	**p**
Total-fruit Index								
Cycling	-0.0000325	0.0000315	1.03	0.303	0.0000211	0.0000271	0.779	0.436
Pregnant	-0.000267	0.0000462	5.78	**<0.001**	-0.000214	0.000049	4.367	**<0.001**
Lactating	-0.00017	0.0000347	4.90	**<0.001**	-0.000116	0.000034	3.412	**0.001**
Vine-fruit Index								
Cycling	-0.0000505	0.0000332	1.52	0.129	0.0000132	0.0000281	0.470	0.638
Pregnant	-0.000268	0.0000454	5.90	**<0.001**	-0.000204	0.0000488	4.180	**<0.001**
Lactating	-0.000181	0.0000336	5.39	**<0.001**	-0.000118	0.0000334	3.533	**<0.001**

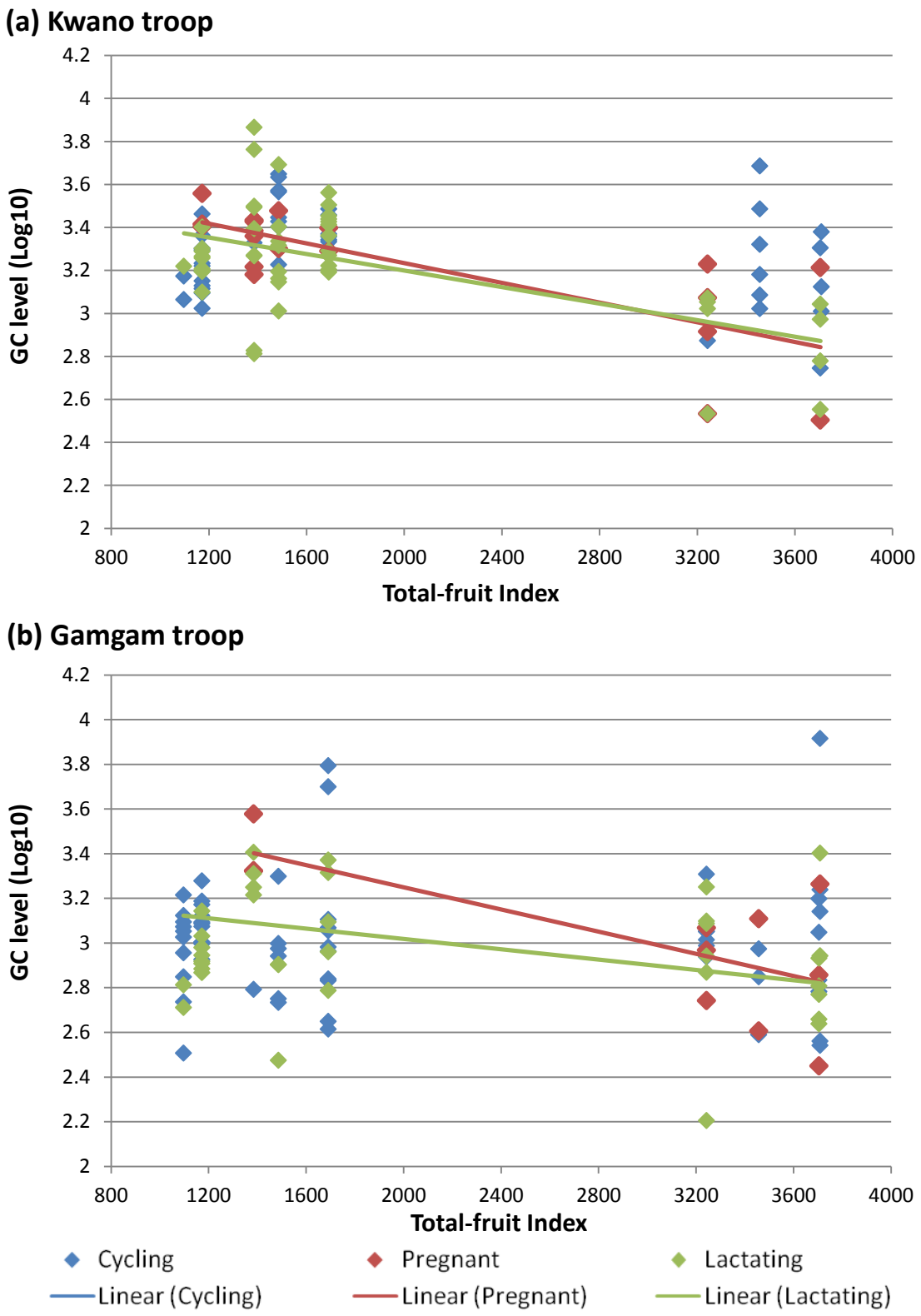

Figure 5.12. Scatter plots showing the effect of reproductive state on the relationships between GC levels and total fruit index for (a) Kwano troop and (b) Gamgam troop. The lines represent the average linear relationships between the two variables for all pregnant and lactating individuals, as predicted by the GLMM.

The addition of the categorical variables troop, season, rank and reproductive state did not significantly improve the fit of the GC 2-factor models containing any of the other weather or fruit index variables (appendix 6c, table A6.ix).

Addition of 30-day rainfall to models

Since 30-day rainfall was strongly related to GC level, this variable was added to the rest of the models in this chapter in order to control for its effect on GC level. Controlling for 30-day rainfall did not reveal any previously unrecognised relationships between GC level and any of the other weather variables (appendix 6c, table A6.xii) and only had a minor effect on the relationships between GC level and the fruit indices. For total- and vine-fruit index, the significant negative relationship with GC level remained for Kwano troop (total: $z=4.29$, $p<0.001$; vine: $z= 6.94$, $p<0.001$) and the relationship for Gamgam troop changed from marginally non-significant to non-significant (total: $z=1.22$, $p=0.221$; vine: $z=1.26$, $p=0.207$). For tree-fruit index the positive relationship with GC level for Kwano troop changed from significant to marginally non-significant ($z=1.94$, $p=0.052$) while the relationship for Gamgam troop remained non-significant ($z=0.338$, $p=0.730$).

5.2.4. Relationships between GC level and energetic status variables

The effects of the energetic status variables, calculated energy intake rate, calculated energy expenditure rate and UCP value, on GC level were assessed in two ways. First, a median GC value for each individual was calculated and this was compared with individual specific median values for calculated energy intake rate, expenditure rate and UCP value. Significant correlations were found between GC and calculated energy intake rate (Pearson's product moment correlation: $r=-0.537$, $n=16$, $p=0.032$, figure

5.13) but not with calculated energy expenditure rate (r=-0.355, n=16, p=0.177) or UCP level (r=-0.173, n=16, p=0.521).

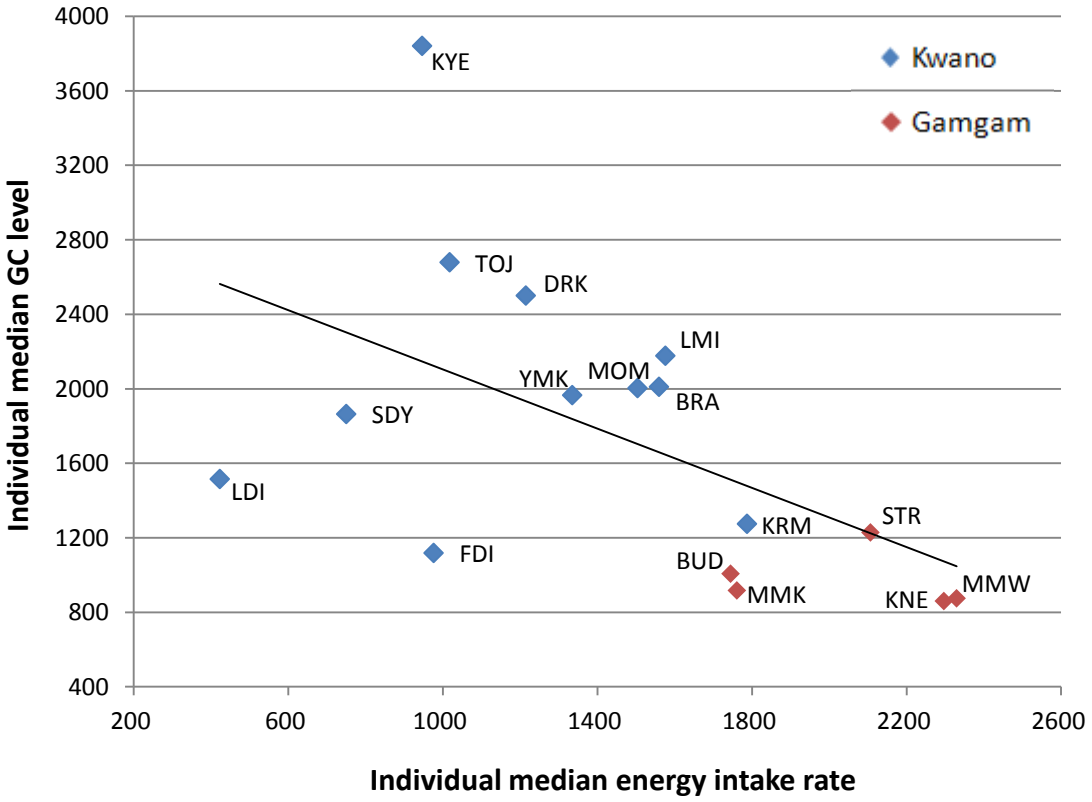

Figure 5.13. Scatter plot showing the relationship between individuals' median GC level (ng/g dry faeces) and their median calculated intake rate (kJ/hr). Line indicates best fitting relationship.

Second, median monthly values of calculated energy intake rate, expenditure rate and UCP value were calculated for each troop and compared to the entire GC dataset (n=211) in the same way as the weather and fruit index variables (section 5.2.3). The effect of the calculated energy rate variables on GC level are considered separately for each troop due to the substantial effect troop has on GC level (figure 5.4), by including the categorical variable troop and its interaction effect in the models. The effects of the categorical variables season, rank and reproductive state on this relationship were also considered separately for each troop.

Median monthly calculated energy intake rate

There was a marginally non-significant interaction effect of troop on the relationship between GC level and median monthly calculated energy intake rate (D=3.55, d.f.=1, p=0.060), with a significant negative relationship for Kwano troop (z=2.63, p=0.009) but no significant relationship for Gamgam troop (z=0.23, p=0.821) (figure 5.14).

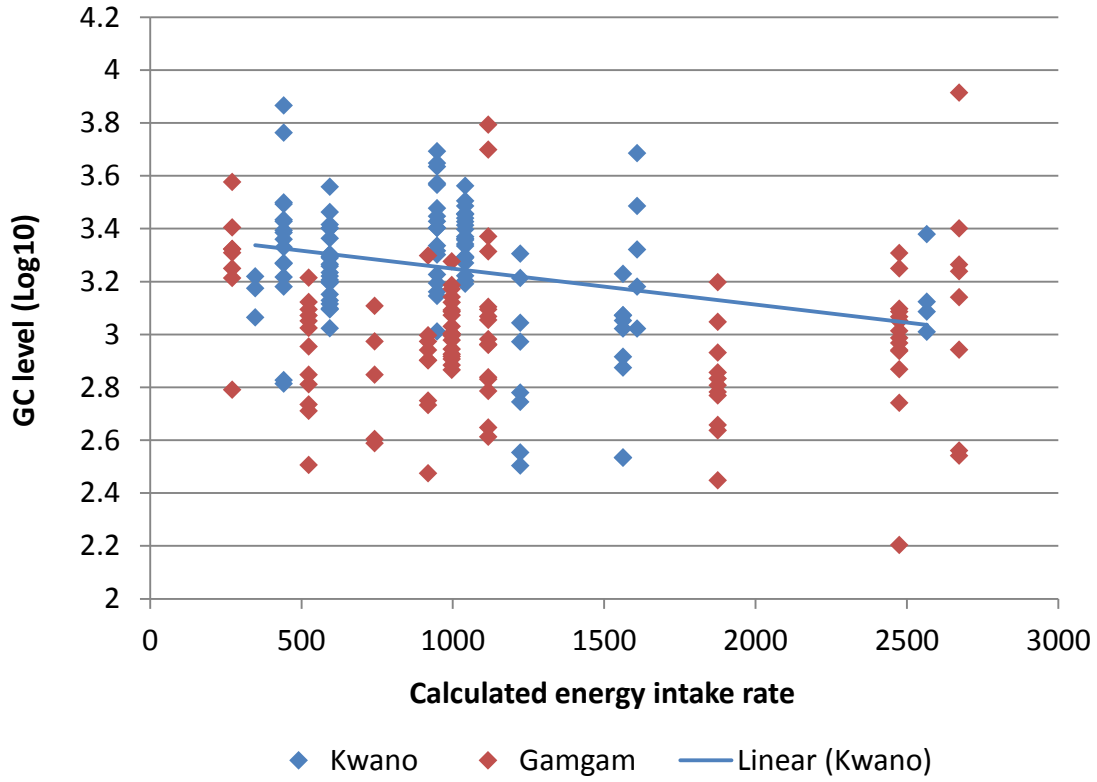

Figure 5.14. Scatter plot showing the effect of troop on the relationship between GC levels (Log_{10}, ng/g dry faeces) and median monthly calculated energy intake rates (kJ/hr). The lines represent the average linear relationships between the two variables for all individuals in each troop, as predicted by the GLMM.

The fit of this model was not significantly improved by the addition of either the season or rank variables, plus interaction effects (appendix 6c, table A6.ix). However, the addition of reproductive state, plus interaction effects, did significantly improve the fit of the model (D=19.96, d.f.=6, p=0.003). For both troops there was a significant negative relationship between GC level and median monthly calculated energy intake rate for pregnant (Kwano: z=4.24, p<0.001; Gamgam: z=2.78, p=0.005) and lactating

animals (Kwano: z=3.77, p<0.001; Gamgam: z=2.11, p=0.035). For cycling animals from Gamgam troop there was a significant positive relationship between the two variables (z=2.37, p=0.018) but for Kwano troop cycling animals there was no significant relationship (z=0.89, p=0.375) (figure 5.15).

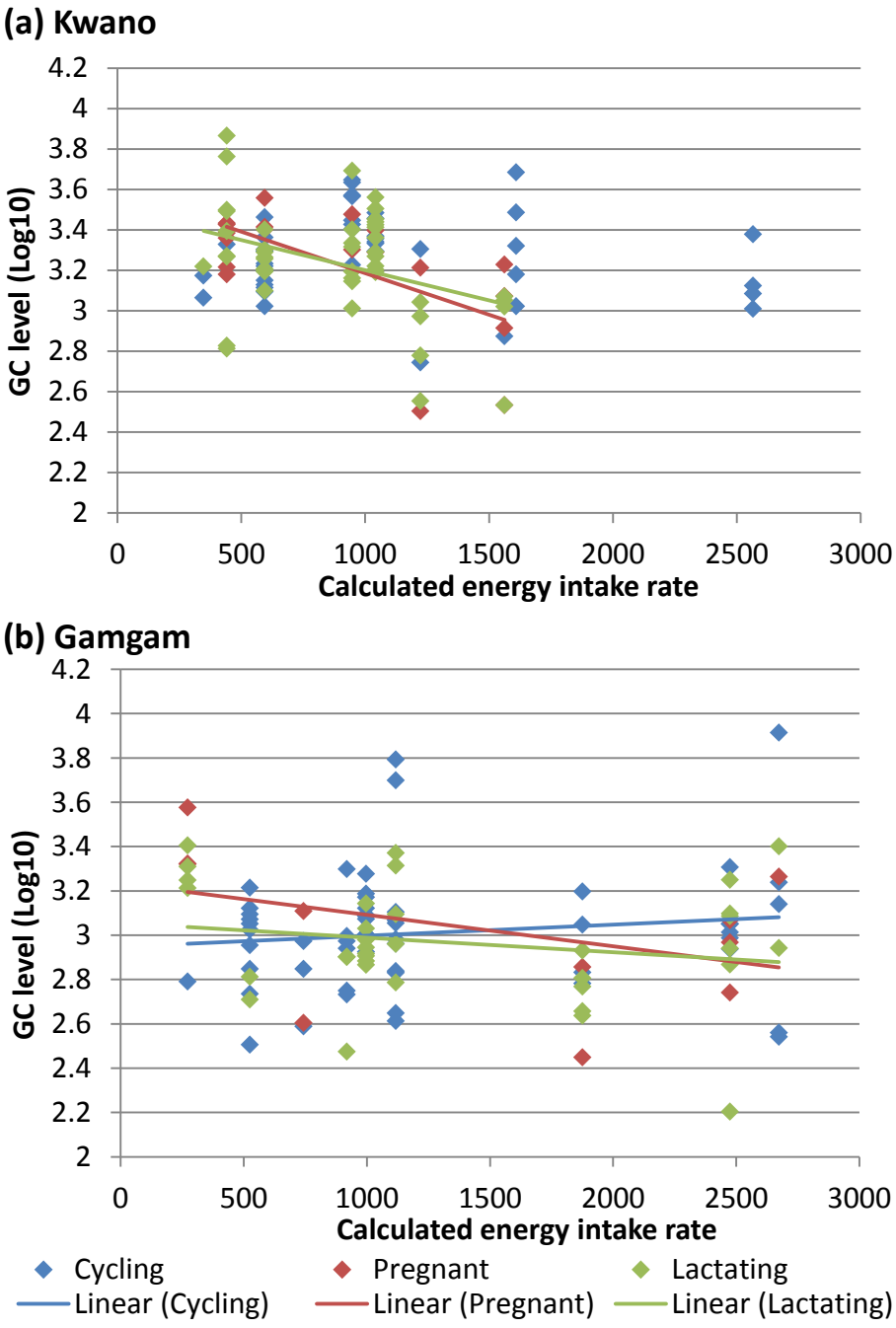

Figure 5.15. Scatter plots showing the effect of reproductive state on the relationship between GC levels (ng/g dry faeces) and median monthly calculated energy intake rate (kJ/hr) for (a) Kwano troop and (b) Gamgam troop. The lines represent the average linear relationships between the two variables for all pregnant and lactating individuals, in each troop, as predicted by the GLMM.

Median monthly calculated energy expenditure rate

There was a significant interaction effect of troop on the relationship between GC level and median monthly calculated energy expenditure rate (D=7.49, d.f.=1, p=0.006), with a, significant positive relationship for Gamgam troop (z=3.44, p<0.001) but no significant relationship for Kwano troop (z=0.62, p=0.534) (figure 5.16).

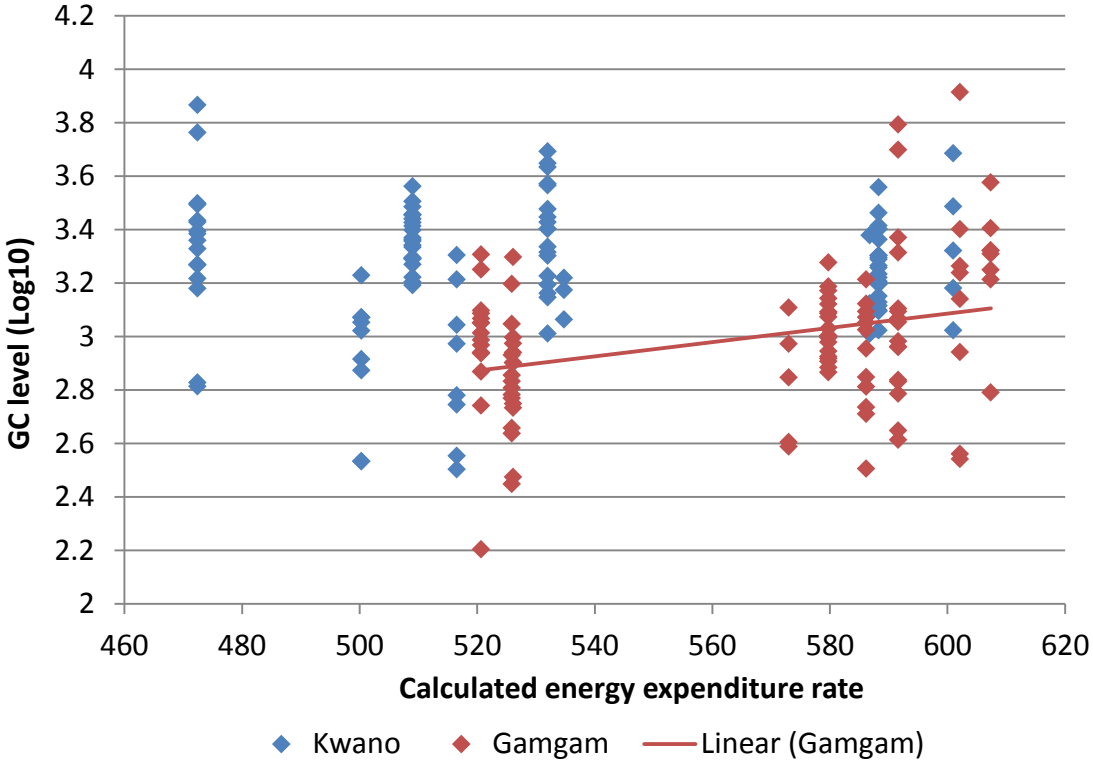

Figure 5.16. Scatter plots showing the effect of troop on the relationship between GC level (ng/g dry faeces) and median monthly calculated energy expenditure rate (kJ/hr). The line represents the average linear relationships between the two variables for all individuals in Gamgam troop, as predicted by the GLMM.

There was a marginally non-significant improvement in the fit of this model on addition of season and its interaction effect (D=7.54, d.f.=3, p=0.057). For Gamgam troop there was a significant negative relationship between GC level and median monthly calculated energy expenditure rate during the wet season (z=4.20, p<0.001) but not during the dry season (z=1.42, p=0.156, figure 4.17) and for Kwano troop there was no significant relationship during either season (dry: z=0.84, p=0.403; wet: z=0.22, p=0.827).

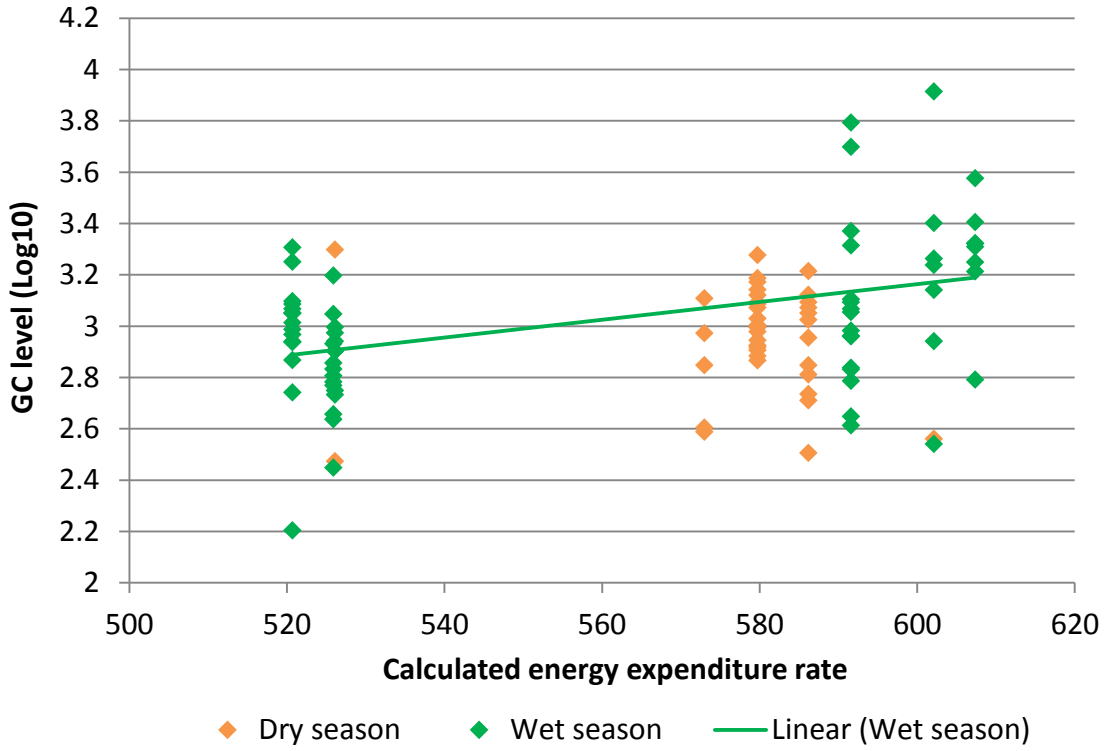

Figure 5.17. Scatter plot showing the effect of season on the relationship between GC level (ng/g dry faeces) and median monthly calculated energy expenditure rate (kJ/hr) for Gamgam troop. The line represents the average linear relationships between the two variables for all Gamgam troop individuals during the wet season, as predicted by the GLMM.

The fit of the GC model containing median monthly calculated energy expenditure and troop was not significantly improved by the addition of either the rank or reproductive state variables, plus interaction effects (appendix 6c, table A6.ix).

Urinary C-peptide level

There was no significant interaction effect of troop on the relationship between GC level and median monthly urinary C-peptide (UCP) level (D=1.54, d.f.=1, p=0.214), with neither troop exhibiting a significant relationship between the two variables (Kwano: z=1.41, p=0.158; Gamgam: z-0.30, p=0.766).

There was a highly significant improvement in the fit of this model on addition of season and its interaction effect (D=19.66, d.f.=3, p<0.001). For both troops there was a

significant positive relationship between GC and median monthly UCP level during the wet season (Kwano: z=3.89, p<0.001; Gamgam: z=3.63, p<0.001) but not during the dry season (Kwano: z=0.25, p=0.802; Gamgam: z=1.55, p=0.121, figure 5.18).

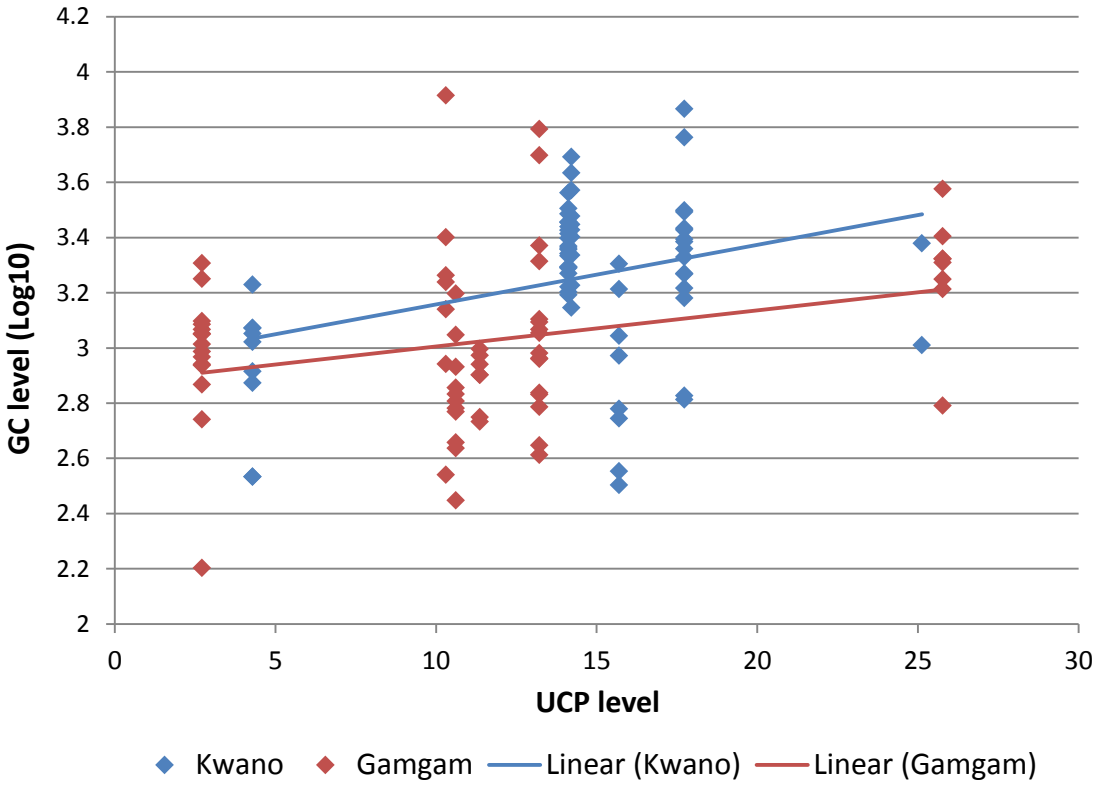

Figure 5.18. Scatter plot showing the effect of troop on the relationship between GC level (ng/mg dry faeces) and median monthly UCP level (ng/mg creatinine) during the wet season for both troops. The lines represent the average linear relationships between the two variables during the wet season for all individuals in each troop, as predicted by the GLMM.

The fit of the GC model containing UCP level and troop was not significantly improved by the addition of either the rank or reproductive state variables plus interaction effects (appendix 6c, table A6.ix).

Effect of 30-day rainfall

The relationship between GC level and the median monthly energetic status variables (intake rate, expenditure rate and UCP level) was examined further by controlling for

the effect of the 30-day rainfall variable, which correlated closely with GC level. The 30-day rainfall variable, and all associated 2-way interaction effects, was added to the following models:

Calculated energy intake rate + troop + interaction effect

When the effect of 30-day rainfall on GC level was controlled for, the negative relationship between median monthly energy intake rate and GC level, which was previously significant for Kwano troop, disappeared ($z=0.18$, $p=0.860$) and for Gamgam troop a significant positive relationship between the variables was revealed ($z=2.39$, $p=0.017$).

Calculated energy intake rate + troop + reproductive state + all 2-way interactions

When the effect of rainfall on GC level was controlled the negative relationships between GC level and median monthly energy intake rate, which were previously significant for pregnant and lactating animals, remained for Kwano troop, although the effect became marginally non-significant (pregnant: $z=1.93$, $p=0.053$; lactating: $z=1.72$, $p=0.086$), but disappeared for Gamgam troop (pregnant: $z=0.61$, $p=0.542$; lactating: $z=0.20$, $p=0.838$). The positive relationship between GC level and intake rate for cycling, Gamgam animals remained significant ($z=2.62$, $p=0.009$).

Calculated energy expenditure rate + troop + interaction

When the effect of 30-day rainfall on GC level was controlled for, both troops demonstrated a significant positive relationship between median monthly energy expenditure rate and GC level (Kwano: $z=3.36$, $p=0.001$; Gamgam: $z=3.84$, $p<0.001$).

Calculated energy expenditure rate + troop + season + all 2-way interactions

When the effect of 30-day rainfall on GC level was controlled for, the positive relationship between median monthly energy expenditure rate and GC level remained significant for both troops (Kwano: z=2.05, p=0.040; Gamgam: z=3.27, p=0.001) and there remained no significant relationship between these variables during the dry season for Kwano troop (z=1.41, p=0.159). However, for Gamgam troop a marginally non-significant positive relationship between GC level and expenditure rate during the dry season was revealed (z=1.81, p=0.071).

UCP level + troop + season + all 2-way interactions

When the effect of 30-day rainfall on GC level was controlled for, the previously significant positive relationship between median monthly UCP level and GC level during the wet season disappeared for both troops (Kwano: z=0.75, p=0.453; Gamgam: z=0.47, p=0.636). Controlling for the effects of rainfall also revealed a marginally non-significant negative relationship between GC and UCP level during the dry season for Gamgam troop only (z=1.87, p=0.062).

5.3. Discussion

5.3.1. Effect of collection time on GC levels

No significant effect of collection time on the GC values of the faecal samples was found. Previous studies have found pronounced circadian patterns of GC secretion with peak levels, for diurnal animals usually occurring in the morning, at the time the animals become active, and with the lowest levels in the evening (Whitten, *et al.*, 1998; Sapolsky, 2000). Faecal metabolites of GC have also been found to follow this pattern, especially in small animals (e.g. rodents and callitrichid primates) where the lag time to excretion of GC metabolites is short (Millspaugh and Washburn, 2004). Many previous

studies of non-human primates have collected faecal samples for GC analysis only in the morning in order to control for this diurnal variation (Crockford *et al.,* 2008; Behie *et al.*, 2010; Brent *et al.,* 2011; Hoffman *et al.*, 2011). However, the results of the present study are consistent with several other studies of Old World primates, which collected faecal samples throughout the day, and also found no effect of collection time on the GC levels (gelada: Beehner and McCann, 2008; Sykes' monkey: Foerster *et al.*, 2010; chacma baboons: Weingrill *et al.*, 2004). These results suggest that diurnal variation in circulating GC levels has no significant effect on levels found in faecal samples for large bodied primates such as baboons.

5.3.2. Effect of troop, rank and reproductive state on GC levels

Troop

Animals from Kwano troop exhibited significantly higher GC levels than animals from Gamgam troop and all Gamgam animals had median GC levels lower than all but one of the Kwano animals. This result is as predicted and is likely to reflect the energetic advantages that the Gamgam troop have over the Kwano troop due to their crop-raiding behaviour. This result is consistent with studies which show that high levels of food availability and quality are associated with low basal GC levels since animals are not under energetic stress (e.g. Muller and Wrangham, 2004; Foerster and Monfort, 2010). This is also consistent with the results from chapter 3 showing that Gamgam troop had a greater energy intake rate and spent less time feeding and more time resting than Kwano troop. These results reflect those from other studies showing a negative relationship between GC level and food intake rate (Pride, 2005), a negative relationship between GC level and resting time (Weingrill *et al.,* 2004) and a positive relationship between GC level and feeding effort (Cavigelli, 1999). The fact that the crop-raiding troop exhibited lower GC levels than the wild-feeding troop also suggests that any effect on

GC levels of crop-raiding behaviour as an acute stressor must be more than outweighed by the energetic benefits. This is in contrast to a study of African elephants, which found elevated faecal GC levels in faecal samples from crop-raiding animals (Ahlering *et al.,* 2011). To my knowledge, this is the first study to provide evidence for a reduction in basal GC level associated with food-enhancement in a free-ranging non-human primate.

Rank

Animals of different social ranks did not differ significantly in their GC levels, either when all individuals were considered together or within troops. There was also no effect of rank on the relationships between GC level and any of the the weather, fruit index or energetic status variables. This result is not as predicted but does reflect the weak effect of rank on activity budgets, energy intake and expenditure rates (chapter 3) and UCP level (chapter 4). As discussed in section 5.1.8, a significant effect of female rank on GC levels is actually rare in studies of free-ranging non-human primates and the results of the present study reflect those of two previous baboon studies that also found no effect of rank on GC level (Weingrill *et al.,* 2004; Crockford *et al.,* 2008). For Old World monkeys living in matrilineal societies it seems likely that the presence of social support prevents low ranking animals from experiencing elevated stress levels, an idea supported by the fact that, among Old World monkeys, low ranking animals do not typically receive more aggression than high ranking animals (Abbott *et al.,* 2003; Weingrill *et al.,* 2004; Crockford *et al.,* 2008).

Reproductive state

GC levels did not vary significantly between the three main reproductive states. However, when the pregnancy data were split into trimesters a significant effect was

revealed, with faecal samples collected from animals in the third trimester of pregnancy exhibiting significantly higher GC levels than animals in all other reproductive states. This result is consistent with the fact that the elevation in the GC levels due to the placental production of corticotrophin-releasing hormone during pregnancy rises to its highest levels just before parturition (Zakar and Mitchel, 1998; Carnegie *et al.,* 2011).

It was predicted that lactating animals would have higher GC levels than cycling animals due to the increased energetic stress and possibility of increased exposure or vulnerability to stress associated with this reproductive state (Weingrill *et al.,* 2004; Emery Thompson *et al.,* 2010; Hoffman *et al.,* 2010), but no evidence was found to support this idea. This result is particularly surprising given the minimal adjustment in energy expenditure exhibited by pregnant animals in this study which, as suggested in section 3.3.2.4, was expected to result in physiological costs to pregnant animals such as elevated GC levels. However, the lack of an effect of lactation on GC levels is consistent with results from two other studies of cercopithecine monkeys (Chacma baboons: Beehner *et al.*, 2005; Engh *et al.,* 2006; Mandrill: Setchell *et al.,* 2008). The variation in the effect of lactation on GC levels between different studies may be related to variation in food availability between habitats, with lactating mothers in low quality habitats experiencing greater energetic stress, or to variation in the risks posed to infants such as the prevalence of infanticide (Weingrill *et al.,* 2004), predation or harassment from other group members (Hoffman *et al.,* 2010), with mothers in more risky situations experiencing greater psychosocial stress. Perhaps the lack of lactation effect at Gashaka in related to the generally productive habitat and/or the low predation pressure (Ross *et al.,* 2011) and the generally low levels of aggression between group members (N. Alberts pers. comm.).

5.3.3. Effect of weather and fruit index variables on GC levels

Weather

GC level was significantly related to 30-day rainfall for both troops but was not related to any other weather variable and did not vary significantly between seasons. This relationship with 30-day rainfall is as predicted and suggests that rainfall acts as a stressor for the Gashaka baboons despite the energetic advantages associated with increased rainfall (chapter 3). Previous studies of other closely related Old World monkeys have demonstrated that environmental stressors can elevate GC levels and that the nature of these stressors varies depending on the local climate e.g. hot temperatures and low rainfall in arid regions (Sapolsky, 1986; Gesquiere *et al.*, 2008), and low temperatures at high latitude (Weingrill *et al.*, 2004) or altitude (Beehner and McCann, 2008). This is the first study, to my knowledge, to demonstrate a positive association between rainfall and stress in a non-human primate (MacLarnon *et al.*, 2010). Previous authors have suggested that rainfall may act as a time constraint on the Gashaka baboons (Higham *et al.*, 2009a) and also increase disease risk resulting in higher infant and perhaps even adult mortality (Ross *et al.*, 2011). The results of the present study support these ideas by showing a link between heavy rainfall and elevated stress levels in the Gashaka baboons. The elevated GC levels observed during periods of heavy rain also give support to the idea, suggested in section 3.2.2.2, that the apparent excess of energy exhibited by the baboons during periods of heavy rain (in terms of low calculated energy balance and expenditure rates) is being spent on hidden, energetic costs i.e. the costs of resisting and combating disease, factors which are associated with elevated GC levels (Chapman *et al.*, 2007; Muehlenbein and Watts, 2010).

Fruit indices

A significant negative relationship was found between GC level and both total- and vine-fruit index for Kwano troop animals during both the wet and dry seasons and this relationship remained when 30-day rainfall was controlled for. This result is as predicted and is consistent with results from previous non-human primate studies that have showed a negative relationship between GC level and fruit availability or consumption (Muller and Wrangham, 2004; Behie *et al.*, 2010; Emery Thompson *et al.*, 2010) and a negative relationship between GC levels and the availability of preferred foods (Foerster and Monfort, 2010). This result also suggests that low fruit availability acts as an environmental stressor for the Kwano baboons. For Gamgam troop the relationship between these two fruit indices and GC level was much weaker. A significant relationship was present during the wet season but this disappeared when the effect of rainfall was controlled for. These results are consistent with the idea that the GC levels of food-enhanced animals are less closely related to environmental stressors than those of entirely wild-feeding animals (Muller and Wrangham, 2004) and also provide further support for the idea, discussed in chapters 3 and 4, that food-enhancement provides a buffer against environmental effects (Bronikowski and Altmann, 1996).

In contrast to the total- and vine- fruit indices, tree fruit index was positively related to GC levels for both troops during the wet season, which is the opposite of what was predicted. However, when the effect of 30-day rainfall was controlled for this relationship became marginally non-significant for Kwano troop and non-significant for Gamgam troop, which suggest that this relationship was mainly due to the fact that tree-fruit index and 30-day rainfall were significantly and positively correlated (Spearman's

rank correlation: r_s=0.458, n= 211, p<0.001). The lack of a negative relationship between GC level and tree-fruit index, in contrast to the strong relationship with vine-fruit index, is probably related to the fact that tree-fruit is available at a fairly constant rate throughout the year whereas vine fruit availability is far more variable (figure 2.2) and is therefore more likely to influence seasonal variation in food intake.

The relationship between GC level and the fruit indices (total and vine) differed between individuals in different reproductive states, with significant negative relationships for pregnant and lactating animals but no significant relationship for cycling animals. This result suggests that animals at energetically expensive life history stages (e.g. pregnancy and lactation) may be more vulnerable to environmental/energetic stressors than animals at less energetically expensive life-history stages (e.g. cycling animals).

5.3.4. Effect of energetic status measures on GC levels

Calculated energy intake rate

Both techniques for examining the relationship between GC levels and calculated energy intake rates revealed significant negative relationships between these variables. Individuals' average GC levels from the entire nine month study period correlated negatively with their average energy intake rate (both troops included) and GC levels were negatively related to the troop's average monthly energy intake rates for Kwano troop but not for Gamgam troop. These results suggest that Kwano troop animals were more energetically-stressed when intake rates were low and also that, considering both troops, individuals with generally low intake rates tend to be more energetically stressed. These results are as predicted and consistent with results from previous studies of non-human primates which have shown negative relationships between GC levels

and both food intake rates (Pride, 2005) and consumption of high quality foods (Muller and Wrangham, 2004; Chapman *et al.*, 2007; Behie *et al.*, 2010; Emery Thompson *et al.*, 2010) as well as the positive relationship found between the fruit indices and GC level in the current study. The lack of relationship for Gamgam troop provides further support for the idea that food-enhancement provides a buffer against the effect of environmental or energetic influences on stress (Bronikowski and Altmann, 1996; Muller and Wrangham, 2004). However, when the effect of 30-day rainfall was controlled for, the negative relationship between GC level and energy intake rate became non-significant and an unexpected positive relationship between these two variables was revealed for Gamgam troop. These results are probably due to the fact that when the faecal sample dataset (n=211), which includes multiple samples from the same day, is considered, there is a weak but significant negative relationship between energy intake rate and 30-day rainfall (Pearson's product-moment correlation: r=-0.211, n=211, p=0.002), which was not present when the focal observation dataset (n=130) was considered (section 3.2.2.2).

Reproductive state had a significant effect on the relationship between GC level and energy intake rate, which was similar to the effect it had on the relationship with total- and vine-fruit index: for pregnant and lactating animals from both troops there was a significant negative relationship between GC level and monthly energy intake rate. For cycling animals from Kwano troop there was no significant relationship between these variables, and for cycling animals from Gamgam troop there was actually a significant positive relationship. These result support the idea that at energetically expensive life-history periods, the impact of energetic stressors becomes greater. When the effect of 30-day rainfall was controlled for the only effect was to change the relationships for

pregnant and lactating animals from significant to marginally non-significant. Again this is most likely related to the negative correlation between intake rate and rainfall.

Calculated energy expenditure rate

No correlation was found between individuals' average GC levels over the study period and their average energy expenditure rates. However, during the wet season there was a significant relationship between GC levels and monthly energy expenditure rates for Gamgam troop and, once the effects of 30-day rainfall were controlled for, this relationship was also observed for Kwano troop. These results are as predicted and are consistent with results from laboratory studies that show a positive link between GC levels and heavy exercise (Tharp, 1975; Girard and Garland, 2002) and studies of free ranging birds showing a positive link between GC levels and travel distance (Angelier *et al.,* 2007 a & b). The present study is the first, to my knowledge, to explicitly compare an estimate of energy expenditure rate and GC levels in a free-ranging non-human primate and to find the predicted positive relationship.

UCP levels

In contrast to predictions, significant positive relationships between GC levels and median monthly UCP levels were found for both troops during the wet season. However, on controlling for the effects of 30-day rainfall this effect disappeared and a trend towards the predicted negative relationship between the variables was revealed, but only for Gamgam troop animals during the dry season. In addition, no correlation was found between individuals' average GC and UCP levels over the study period.

Although a negative relationship between GC and UCP level was predicted, due to fact that energetic stress should be characterised by high GC and low UCP levels, the relationship between GC and UCP levels is complicated. As discussed in section 5.1.3,

the action of GCs and insulin can be both synergistic and antagonistic and the production of one compound can trigger the production of the other (Sapolsky *et al.*, 2000). This complex relationship may account for the varied and generally weak relationship found between GC and UCP level in this study.

5.4. Summary of results in relation to the original hypotheses

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

- Kwano troop exhibited significantly higher GC levels than Gamgam troop, consistent with the idea that crop-raiding reduces energetic stress.
- The GC levels of Kwano troop were more closely related to food availability and energy intake rate than were those of Gamgam troop, consistent with the idea that food-enhancement provides a buffer against the effect of environmental or energetic influences on stress.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary

- No evidence for an effect of dominance rank on the Gashaka baboons' GC levels was found.
- GC levels were elevated in the third trimester of pregnancy, consistent with understanding of placental hormone production.
- Negative relationships between GC levels and calculated energy intake rates and positive relationships between GC levels and calculated energy expenditure rates are presented and discussed.
- Evidence is presented which supports the idea that animals at energetically expensive life-history stages (i.e. pregnancy and lactation) are more vulnerable to energetic stressors than other animals.

- Little evidence for a simple relationship between GC and UCP levels was found.

Hypothesis 3: The baboons' condition will co-vary throughout the year in response to variation in weather patterns and food availability.

- GC levels were significantly and positively related to the cumulative rainfall over the previous 30 days. The importance of rainfall as a climatic stressor, for the Gashaka baboons, is discussed.
- Negative associations between GC levels and fruit availability were found, for Kwano troop only.

Chapter 6

PROGESTERONE LEVELS AND REPRODUCTIVE SUCCESS

6.1. Introduction

In this chapter the relationship between the variables considered to represent individual energetic status and general condition, which were developed and discussed in the previous three chapters (i.e. calculated energy intake rate, expenditure rate, urinary C-peptide (UCP) and glucocorticoid (GC) levels) will be compared with measures of reproductive condition (progesterone levels) and reproductive success (birth and infant survival rates). The aim of this final results chapter is, therefore, to help to elucidate the link between short term measures of individual energetics and health and longer-term fitness.

6.1.1. Progesterone

Progesterone is a reproductive hormone present in all mammalian species and is the major secretion product of the ovaries. It has several roles in female reproduction including influencing the release of gonadotrophins (including luteinizing hormone, LH); stimulating uterine secretion during the follicular phase, which facilitates implantation of fertilised ova; and maintaining pregnancy (Norris, 2007). In the normal primate reproductive cycle, progesterone is produced at low levels during the pre-ovulatory, follicular phase when the follicles are developing under the influence of gonadotrophins. Progesterone rises after ovulation, due to its production by the corpus luteum in the ovary, peaks during the middle of the luteal cycle and then, if fertilisation does not occur, decreases again until it reaches follicular levels at the start of the next cycle (figure 6.1). This drop in progesterone allows the production of gonadotrophins and therefore stimulation of follicular development (Norris, 2007). If fertilisation and

implantation are successful progesterone levels rise further and are maintained at well above luteal phase levels throughout pregnancy, due to placental production, rising even higher just before birth and dropping rapidly following birth and the detachment of the placenta (Albrecht and Townsley, 1976; Norris, 2007).

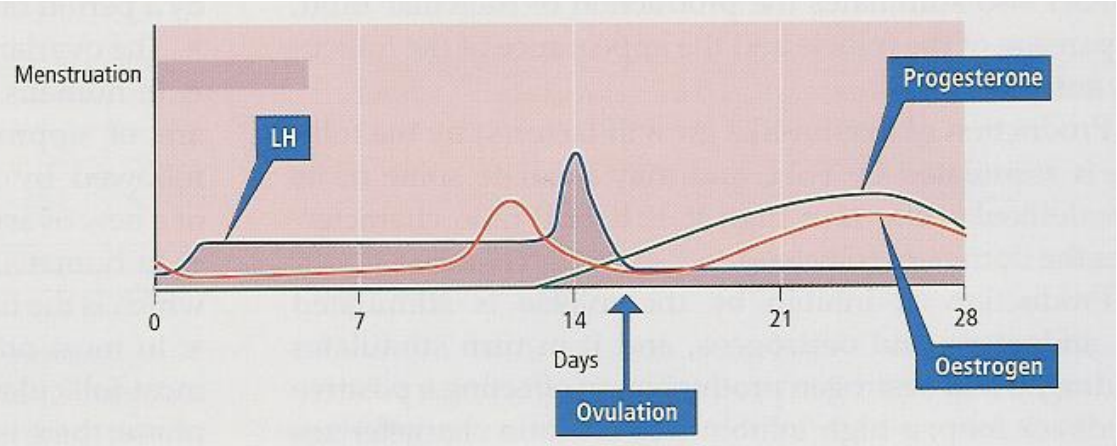

Figure 6.1. Diagram showing the variation in important hormones (luteinizing hormone (LH), oestrogen and progesterone) during the typical primate ovarian cycle. Taken from Johnson and Everitt (2000).

In addition to the expected variation during the reproductive cycle, an individual primate's progesterone levels can be affected by other factors including energy balance, stress and exogenous sources (Ellison, 1990; Higham *et al.*, 2007).

Progesterone, energy balance and stress

The relationship between progesterone and energy balance is complex, with some evidence pointing towards a positive association between progesterone levels and energy balance and other evidence suggesting the opposite relationship. A positive association between progesterone levels and energy balance is well documented in human studies, with progesterone levels known to vary with age, nutritional status, diet and energy expenditure (Ellison, 1990; Bentley *et al.*, 1998; Ellison, 2003). Specifically, moderate energetic stressors, including moderate weight loss, exercise and

supplemented breastfeeding, can result in mild ovarian suppression characterised by lowered progesterone levels during the luteal phase, and associated with increased chance of conception failure and spontaneous abortion (Ellison, 1990; Bentley *et al.*, 1998; Ellison, 2003). Significant positive correlations between luteal progesterone levels and conception probability have also been found for chimpanzees (Emery Thompson, 2005), gorillas (Nadler and Collins, 1991) and baboons (Wasser, 1996). Progesterone levels are therefore often considered as a proxy for conception probability and hence likely reproductive success (Ellison, 1990; Ellison, 2003).

Most of the research into the relationship between nutrition and ovarian hormone levels comes from domestic livestock. For example, several studies have demonstrated that a restricted diet can lead to a reduction in circulating, luteal progesterone levels in cattle (Donaldson *et al.,* 1970; Gombe and Hansel, 1973; Imakawa *et al.,* 1983). Another study investigating the effect of restricted diet on progesterone in cattle found that animals fed a restricted diet had significantly lower progesterone content and concentration in their ovaries than animals fed a control diet but that, in contrast to the results of the other studies, circulating progesterone levels were consistently higher for the animals on the restricted diet. These elevated progesterone levels occurred alongside reductions in weight and fat mass of the food restricted animals (Dunn *et al.,* 1974). An inverse relationship between nutritional status and circulating progesterone levels has also been found in domestic sheep during both the luteal phase of the oestrus cycle (Williams and Cumming, 1982) and during early pregnancy (Parr, 1992). The negative relationship between nutritional status and progesterone level evident in several studies of domestic livestock, including cattle, sheep and pigs, appears to be due mainly to the fact that increasing the mass of food an animal consumes increases the blood flow to the liver and therefore the metabolic clearance rate of hormones such as progesterone,

resulting in lowered circulating levels (Williams and Cumming, 1982; Parr *et al.,* 1987; Parr, 1992; Prime and Symonds, 1993; Sangsritavong *et al.,* 2002). In this way it appears that these domestic animals are eating 'too much' food which is having a detrimental effect on their ovarian hormone levels and ultimately their reproductive success, a phenomenon which has been linked to reduced pregnancy success in over-fed sheep (Parr *et al.,* 1987) and to a substantial decline in the reproductive efficiency of dairy cows over the last few decades (Sangsritavong *et al.,* 2002).

Only a few studies have investigated the link between energetics and progesterone levels in free-ranging animals. Female chimpanzees living in food rich habitats have been found to exhibit higher progesterone levels than those living in food poor habitats alongside shorter inter-birth intervals and higher infant survival (Emery Thompson *et al.,* 2007) and in African elephants, progesterone levels were shown to decrease during the dry season alongside body condition, as food and water availability decreased (Foley *et al.,* 2001). The results of these studies suggest a positive relationship between progesterone levels and energy balance but others support the opposite relationship. For example, in a study of free-ranging yellow baboons, Wasser (1996) demonstrated that conceptive cycles were characterised by significantly higher luteal phase progesterone levels compared to non-conceptive cycles, supporting the idea that high luteal phase progesterone levels facilitate successful implantation. However, when only conceptive cycles were considered, high ranking baboons exhibited significantly lower luteal progesterone levels than low ranking baboons and wet season luteal progesterone levels were significantly lower than dry season levels. This means that animals likely to be experiencing the least favourable energetic conditions, i.e. low ranking animals during the dry season, exhibited the highest luteal progesterone levels during conceptive cycles. In contrast, season and rank had no effect on the luteal progesterone levels of

non-conceptive cycles, although they did influence the likelihood of conceiving. Wasser (1996) suggested that during unfavourable energetic conditions the luteal phase progesterone threshold for successful implantation actually increases, thereby reducing the probability of being pregnant and producing an infant under energetically stressful conditions, which might otherwise lead to wasted investment, if the offspring dies, and reduced future reproductive success. Based on the results of this study, animals under greater energetic stress at Gashaka may be expected to exhibit higher progesterone levels, at least during conceptive cycles, than those experiencing more favourable energetic conditions.

Negative associations between energy balance and progesterone levels have been demonstrated in studies of deer. Under-nourished females exhibited elevated progesterone levels, relative to well fed females, during both the pre-breeding period (red deer: Cook, *et al.,* 2001) and during pregnancy (white-tailed deer: Verme *et al.,* 1965; Bahnak *et al.,* 1979; Plotka *et al.,* 1983). In these cases the authors suggest that this elevation may be due to the production of adrenal progesterone as a result of stress (Cook, *et al.,* 2001; Plotka *et al.,* 1983). It has been suggested that progesterone production by the adrenal gland during times of stress can act to inhibit reproduction in cycling females, by inhibiting LH production, ovulation and fertilization processes, while increases during pregnancy can prevent spontaneous abortion, due to its role in maintaining pregnancy, which can be triggered by high levels of glucocorticoids (GCs) (Plotka *et al.,* 1983). Results from African elephants also support this notion with progesterone and GC levels co-varying and showing a close correlation throughout pregnancy (Foley *et al.,* 2001).

The relative importance of adrenal progesterone has been demonstrated in studies of rats where production and secretion rate of progesterone by the adrenal gland of unstressed animals was similar, in magnitude, to that of the ovaries. In addition, exposure to stressors was shown to increase adrenal but not ovarian progesterone content and secretion (Fajer *et al.*, 1971). Adrenal progesterone production due to stress could also provide a possible explanation for the results of the study in domestic cows, which demonstrated that food restricted animals had a reduced ovarian progesterone content, relative to control fed animals, but exhibited higher circulating progesterone levels (Dunn *et al.*, 1974).

Progesterone production during stress is also likely to occur in primates: adrenal progesterone is thought to play a role in LH secretion in rhesus macaques (Xiao *et al.*, 1997) and elevated progesterone levels following handling by people have been attributed to adrenal progesterone production in bonnet macaques (Lasley *et al.*, 1974). In contrast, though, stress has been shown to result in an immediate reduction in plasma progesterone levels alongside increases in plasma GCs in Guinea baboons (Albrecht *et al.*, 1978). Declines in progesterone levels as a result of stress have also been demonstrated in several laboratory studies of non-human primates (O'Connor *et al.*, 2011). For example, luteal phase progesterone levels of long-tailed macaques have been shown to decline as a result of both energetic and psychosocial stressors (Williams *et al.*, 2007). In rhesus macaques various stressors have induced lowered luteal phase progesterone levels alongside raised glucocorticoid levels (Xiao *et al.*, 1999; Xiao *et al.*, 2002) and lowered luteal phase progesterone levels as a direct result of exogenous glucocorticoid treatment has been demonstrated (Hayashi and Moberg, 1990). However, these results are not universal with other studies of non-human primates finding no association between glucocorticoid and progesterone levels (O'Connor *et al.*, 2011).

Higham's (2006) study of the Gashaka baboons found that Kwano animals exhibited higher levels of progesterone in faecal samples than the food-enhanced Gamgam animals, which, the author suggested, may be due to the poorer nutritional status of the Kwano animals. This result is therefore consistent with those studies of ungulates, which demonstrated a negative relationship between nutritional status and circulating progesterone levels (Verme *et al.,* 1965; Donaldson *et al.,* 1970; Gombe and Hansel, 1973; Dunn *et al.,* 1974; Bahnak *et al.,* 1979; Williams and Cumming, 1982; Imakawa *et al.,* 1983; Plotka *et al.,* 1983; Parr, 1992; Cook *et al.,* 2001).

6.1.2. Progesterone and *Vitex doniana* consumption

During a previous study of the Gashaka baboons' reproductive hormones, major seasonal peaks in progesterone levels, regardless of female reproductive state, were identified, which coincided with the consumption of the new leaves and fruit from the species *Vitex doniana*. Subsequent analysis of the fruit and leaves of this species revealed high levels of progesterone-like compounds (Higham *et al.,* 2007). Other species from the *Vitex* genus have been found to affect human reproductive function (Weiss, 1988; Sliutz *et al.,* 1993; Berne *et al.,* 1998; Chevallier, 2001; Liu *et al.,* 2001; Liu *et al.,* 2004) and have been implicated in unusually high progesterone levels of chimpanzees (Emery Thompson *et al.,* 2008) and Phayre's leaf monkeys (Lu *et al.,* 2009). Higham *et al.* (2007) also demonstrated that *V. doniana* consumption, and the baboons' elevated progesterone levels, coincided with a reduction in the sexual swellings of cycling females, which are indicative of ovulation and associated with increased association and copulation with males. The authors therefore suggested that *V. doniana* acts as both a physiological contraceptive, by simulating the physiological effects of pregnancy (i.e. high progesterone levels), and a social contraceptive, by reducing sexual swellings and therefore male mating interest (Higham *et al.*, 2007).

6.1.3. Reproductive rates: relationships with energetics, stress and progesterone

Individual lifetime fitness is determined by the number of offspring an individual produces, which survive to reproductive maturity. Life-history studies, therefore, often use birth rates, inter-birth intervals (IBIs) and offspring survival rates as proxies for an individual's lifetime fitness and compare these measures to environmental, social and energetic factors in order to determine which of these factors influence and shape life-history characteristics and individual or population success (Altmann, 1991). As discussed in section 1.2.1 of this thesis, a female's energetic status is likely to have a profound effect on her reproductive performance. In humans, the links between a woman's energy balance and both her conception probability and length of lactational amenorrhea, two important determinants of IBIs, are well established, as is the link between energetic condition during pregnancy and pregnancy success (Ellison, 1990; Ellison, 2003). In non-human primates evidence for these links comes from comparisons of captive and free-ranging animals (Bentley, 1999; Garcia *et al.,* 2009), comparisons of animals occupying different quality habitats (Altmann and Alberts, 2003b; Emery Thompson *et al.,* 2007), links between body mass and reproductive success (Bercovitch, 1987; Garcia *et al.,* 2006; Garcia *et al.,* 2009) and comparisons of food-enhanced vs. wild-feeding animals (Mori, 1979; Sugiyama and Oshawa, 1982; Mori *et al.,* 1997; Altmann and Alberts, 2003b). Particular measures of energy intake during infancy have also been found to predict accurately various measures of lifetime fitness in wild baboons, including total number of infants and yearlings produced (Altmann, 1991).

Stress and reproductive success

Negative effects of stress, or more specifically elevated GC levels, on maternal behaviour have been demonstrated in both humans and non-human primates (Bahr *et*

al., 1998; Bardi *et al.,* 2003, 2004; Krpan *et al.,* 2005; Saltzman and Abbott, 2009). However, positive associations between GC levels and maternal attention have also be found (Fleming *et al.,* 1987; Fleming *et al.,* 1997) and the exact relationship between GC levels and maternal behaviour seems to depend on the female's previous maternal experience (Saltzman and Abbott, 2009). This negative relationship between stress and maternal behaviour can apparently translate into reductions in reproductive success. For example in a captive colony of common marmosets a period of increased stress corresponded with increases in maternal aggression towards infants and reduced infant survival rates (Johnson *et al.,* 1991).

6.1.4. Predictions

The relationships between progesterone levels, energetic status, stress and reproductive performance are clearly complex. It is therefore difficult to formulate simple predictions about the relationships between these factors from the study hypotheses. For this reason, the direction of some of the predictions is left open:

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

Predictions:

a. Kwano troop will have higher progesterone levels than Gamgam troop (based on previous findings: Higham, 2006), although this may vary between reproductive states.
b. Kwano troop will have lower birth and yearling production rates than Gamgam troop.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary.

Predictions:

a. Progesterone levels may differ between animals of different ranks, although the direction of the effect is hard to predict and may depend on reproductive state.
b. Reproductive rates will be higher for higher ranking females
c. Progesterone level may be related to calculated energy intake rate, expenditure rate and urinary C-peptide level, although the direction of the relationship is hard to predict and may depend on reproductive state.
d. Progesterone levels will be positively related to GC levels (based on previous studies that have linked elevated progesterone levels to stress e.g. Lasely *et al.*, 1978).
e. Reproductive rates will be higher amongst animals with higher average energy intake rates and lower average energy expenditure rates.
f. Reproductive rates will be higher amongst animals with higher average urinary C-peptide levels.

6.2. Results

For methods of faecal sample collection and analysis for PdG (progesterone metabolite) content see chapter 2, sections 2.2.2 and 2.3.1, respectively. As in chapter 5, a 2-day excretion lag is used; analysis using a one day excretion lag did not affect the reported relationships.

A total of 211 PdG vales are used in the following analyses. The data range from 415 to 44999 ng of PdG per g dry faecal mass and are right skewed. Table 6.1. gives the number of samples and median PdG values for each individual and the two troops.

Table 6.1. Median and range of PdG concentrations for individual focal animals and troops.

Focal ID	Median PdG concentration[1] (range in brackets)	n
BRA	2047 (13831)	11
DRK	24939 (37830)	7
FDI	4717 (27396)	8
KRM	4611 (34525)	8
KYE	11739 (40610)	6
LDI	3759 (12918)	9
LMI	5633 (15094)	12
MOM	4661 (24112)	11
SDY	3132 (13495)	13
TOJ	5546 (22738)	11
YMK	2797 (30197)	12
Kwano[2]	5319 (44336)	108
BUD	2665 (11636)	17
KNE	4305 (39305)	24
MMK	4868 (21751)	25
MMW	1710 (22978)	11
STR	2955 (13910)	26
Gamgam[2]	3400 (39393)	103

1. PdG concentration in ng/g dry faecal mass
2. Troop values are medians from all PdG values for that troop

Effect of collection time on PdG concentration

There was no significant correlation between the time of day that a sample was collected and PdG level (Spearman's rank correlation: r_s=0.058, n=224 (including replicate samples), p=0.388, figure 6.2). This result is in contrast with a study of common marmosets, which showed a peak in progesterone excretion in the afternoon (Sousa and Zeigler, 1998) and suggests that diurnal variation in circulating PdG levels has little effect on levels found in faecal samples for large bodied primates such as baboons.

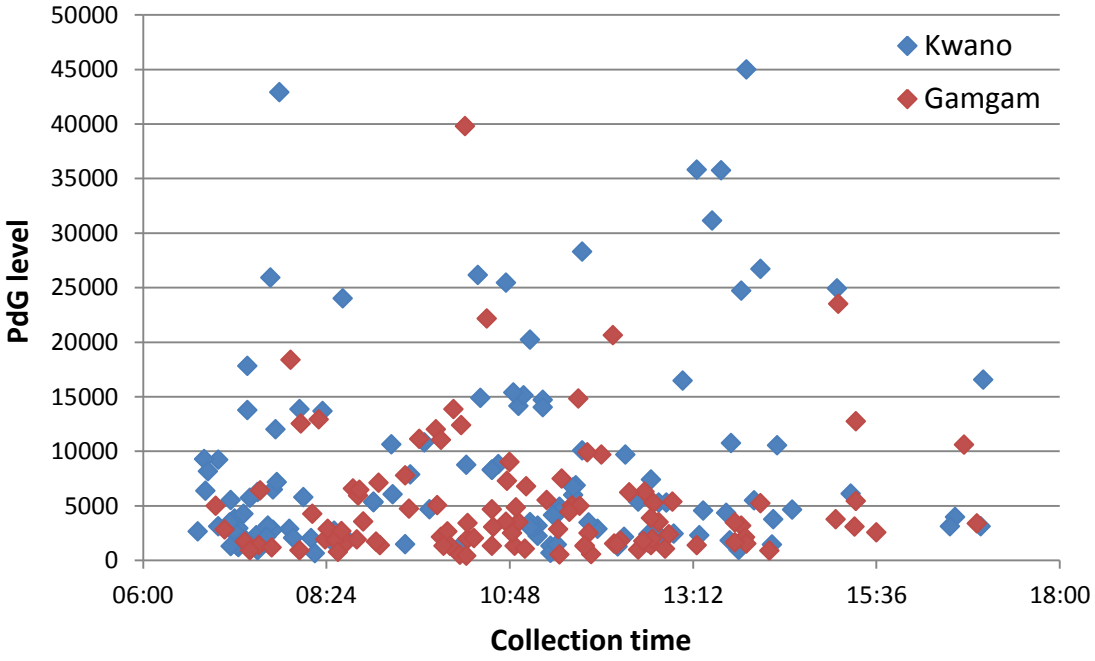

Figure 6.2. Scatter plot showing the variation in PdG levels (ng/g dry faeces) with faecal sample collection time.

Effect of faecal sample mass on PdG level

There was a significant negative correlation between faecal sample mass and PdG level (Spearman's rank correlation: r_s=-0.149, n=224, p=0.026, figure 6.3). However, this correlation appears to be largely driven by two low mass, high PdG values from pregnant animals, the removal of which causes the correlation to become marginally non-significant (r_s=-0.130, n=222, p=0.053), and the fact that none of the highest weight samples (> 1.1g) were from pregnant animals (figure 6.3). These two data points were not considered to be outliers and so were included in all further analyses.

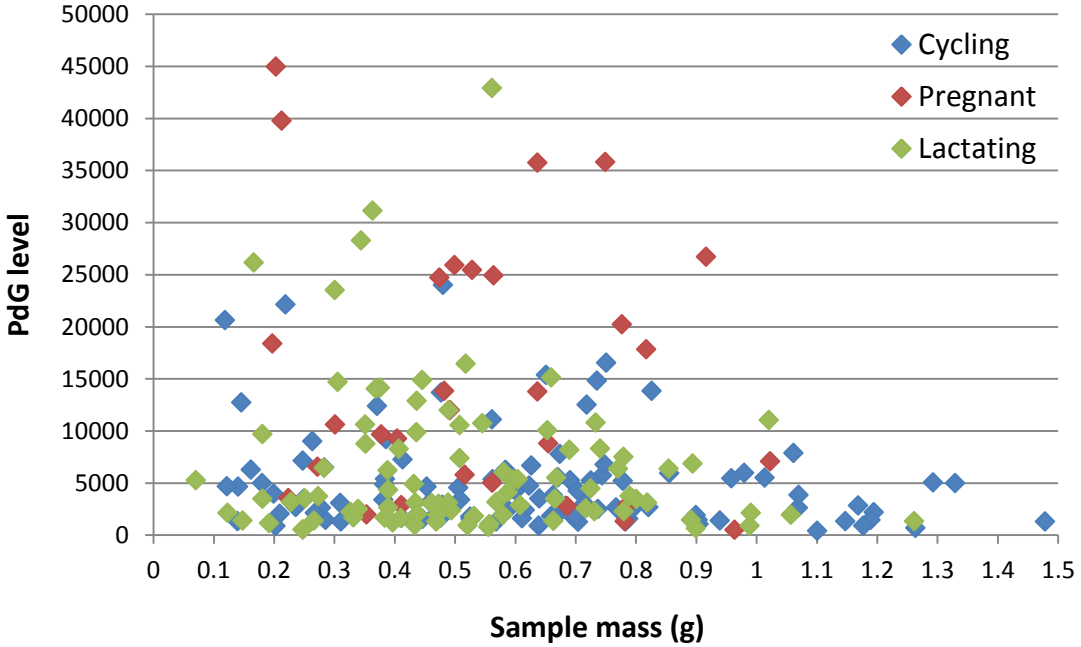

Figure 6.3. Scatter plot showing variation in PdG levels (ng/g dry faeces) with faecal sample mass (g) for focal animals in different reproductive states.

6.2.1. *Vitex doniana* availability during the current study

Previous analysis of the Gashaka baboons' diet revealed that *V. doniana* fruit acted as a staple food (contributed to greater than 1% of total foraging time) during the month of August for both troops and the new leaf constituted a staple food during January for Kwano troop only (Warren, 2003). Since the field work for the current study took place between March and December only the consumption of *V. doniana* fruit was considered. A re-analysis of Warren's (2003) data revealed that *V. doniana* fruit were consumed exclusively during August, September and October, contributing to up to 4.1% of total foraging time for Kwano troop and 2.9% of foraging time for Gamgam troop during this period (Higham, 2006).

During the current study consumption of *V. doniana* fruit by the Gashaka baboons was observed between the 26th August and the 17th September and accounted for 5.3 and 2.9% of total feeding time for Kwano and Gamgam troops respectively during these two months (table 6.2).

Table 6.2 Timing of the consumption of *Vitex doniana* fruit by the Gashaka baboons

	Period of observed *V. doniana* fruit consumption		% total feeding time spent on *V. doniana* fruit			Total number of *V. doniana* feeding instances recorded in scan samples		
	First record	**Last record**	**August**	**September**	**Total**	**Adult females**	**Other animals**	**Total**
Kwano	26/08/09	10/09/09	2.92	6.40	5.30	20	27	47
Gamgam	31/08/09	17/09/09	0.83	4.17	2.92	3	7	10
Both troops	26/08/09	17/09/09	2.54	6.14	4.94	23	34	57

Since it is likely that some instances of *V. doniana* consumption were missed, the PdG levels of samples collected during different months, from cycling, pregnant and lactating animals, were compared, with the assumption that unusually high PdG levels at any time during the period when *V. doniana* fruit might be available (i.e. beginning of August until the end of October: Higham, 2006) would indicate *V. doniana* consumption. A visual inspection of the data (figure 6.4) indicated that the PdG levels of animals in all three reproductive states appeared to be substantially elevated during August and September, which suggests that it is during these two months of the study period that *V. doniana* consumption was influencing the baboons' progesterone levels. It is worth noting here that no data were collected in July 2009 so it is unknown whether *V. doniana* fruit was being consumed and whether PdG levels were elevated during this month.

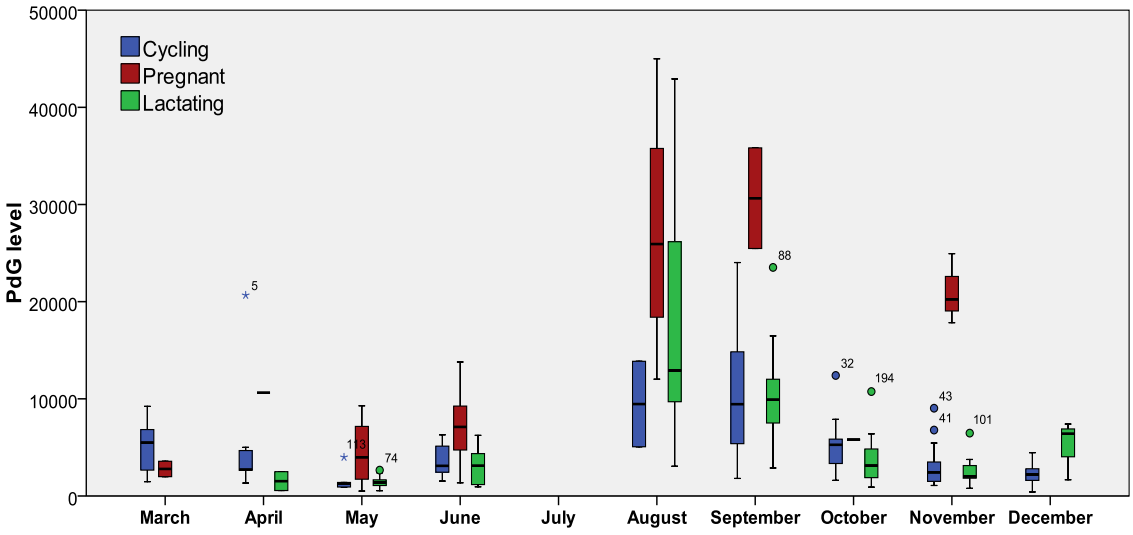

Figure 6.4. Box-plot showing the effect of calendar month on the PdG levels (ng/g dry faeces) of cycling, pregnant and lactating animals during the 2009 study period.

Further support for this supposition came from a comparison of individual animals' median PdG levels from before, during and after this period of presumed *V. doniana* consumption. Median PdG levels were calculated for each individual for each reproductive state, in order to control for the known influence of reproductive state on progesterone levels. Each female contributed only one set of values, even if she was in more than one reproductive state during the *V. doniana* consumption period. For example, if a female gave birth during the period of proposed *V. doniana* consumption median values could be calculated from both her pregnant PdG levels, before and during the period of *V. doniana* consumption, and her lactating PdG levels, from during and after the period of *V. doniana* consumption. In this case one of these sets of results would be removed. Selection of which set of values to remove was based on creating a sample which represented all three reproductive states equally. The final paired t-test was based on pairs of values from 15 of the 16 focal animals with five each from cycling, pregnant and lactating animals.

Median PdG values of individual females were significantly greater during the period of *V. doniana* fruit availability compared to the proceeding period (Paired t-test: $t=-5.95$, $d.f.=10$, $p<0.001$) and subsequent period ($t=-4.94$, $d.f.=11$, $p<0.001$) (figure 6.5). There was no significant difference between females' median PdG levels in the periods before and after *V. doniana* availability ($t=-1.35$, $d.f.=7$, $p=0.220$).

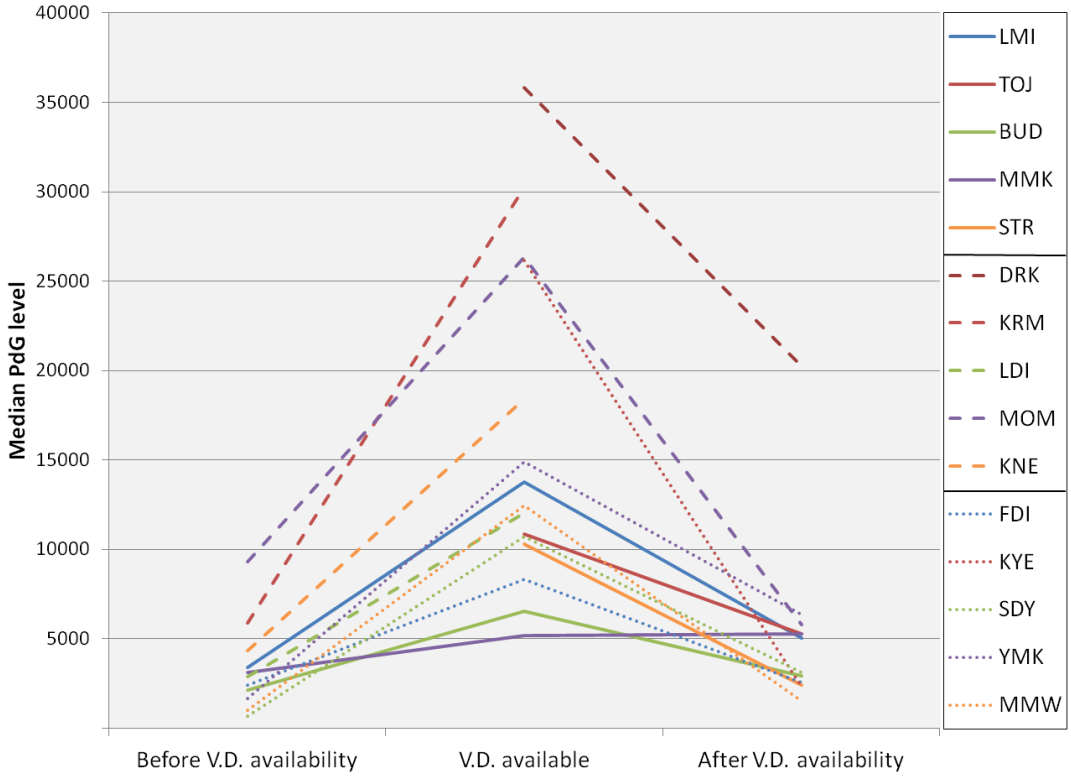

Figure 6.5. Line chart showing within individual variation in PdG levels (ng/g dry faeces) before, during and after the period of proposed *Vitex doniana* (V.D.) consumption. Solid lines indicate cycling values, dashed lines indicated pregnant values and dotted lines indicate lactating values

These results suggest that *V. doniana* consumption during the study period had the greatest effect on PdG levels during the months of August and September, although its effect in July is unknown. For this reason a '*V. doniana* availability' variable was added to the models in the following section, in order to control for the effects of potential *V. doniana* consumption on PdG levels while investigating the effects of other potentially influential variables. PdG values from samples collected during August and September were assigned to the category '*V. doniana* available' and values from samples collected in the other 7 months were assigned to the category '*V. doniana* not available'.

6.2.2. Effect on PdG levels of categorical variables: *V. doniana* availability, troop, rank, reproductive state and pregnancy stage

The effect of the categorical variables (*V. doniana* availability, troop, rank and reproductive state) on PdG levels were assessed by building GLMMs with Log_{10} transformed PdG data (n=211) as the dependent variable and with faecal sample number and ID fitted as random effects. A fifth categorical variable, pregnancy stage, was created by splitting the values from pregnant females into those collected during the first (P1), second (P2) and third (P3) trimesters. The five categorical variables were added to the PdG 2-factor null model (which contained only the two random factors) in turn as fixed effects. Full results of statistical analyses relating to all the models in this chapter are presented in appendix 6d, table A6.xiii-xix.

The fit of the GC 2-factor null model was significantly improved by the addition of *V. doniana* availability ($D=121.40$, d.f.=1, $p<0.001$), troop ($D=121.40$, d.f.=1, $p<0.001$), reproductive state ($D=24.20$, d.f.=2, $p<0.001$) and pregnancy stage ($D=25.52$, d.f.=3, $p<0.001$) but not with the addition of rank ($D=0.79$, d.f.=2, $p=0.130$). PdG levels were significantly higher in the two months when *V. doniana* fruit was available than in the other months ($z=12.90$, $p<0.001$, figure 6.6a); were significantly higher for Kwano troop than Gamgam troop ($z=3.00$, $p=0.003$, figure 6.6b); and were significantly higher in samples from pregnant animals than from samples from cycling ($z=5.11$, $p<0.001$) and lactating ($z=4.12$, $p<0.001$) animals (figure 6.6c). The PdG levels of pregnant females in all three trimesters were higher than non-pregnant females, although this difference was marginally non-significant for 1st trimester samples, but there was no significant difference in PdG level between trimesters (figure 6.6d, table 6.3). For this reason, pregnancy stage was not used in the following analyses and instead the PdG

values from all trimesters were lumped together within the 'pregnant' category of the 'reproductive state' variable.

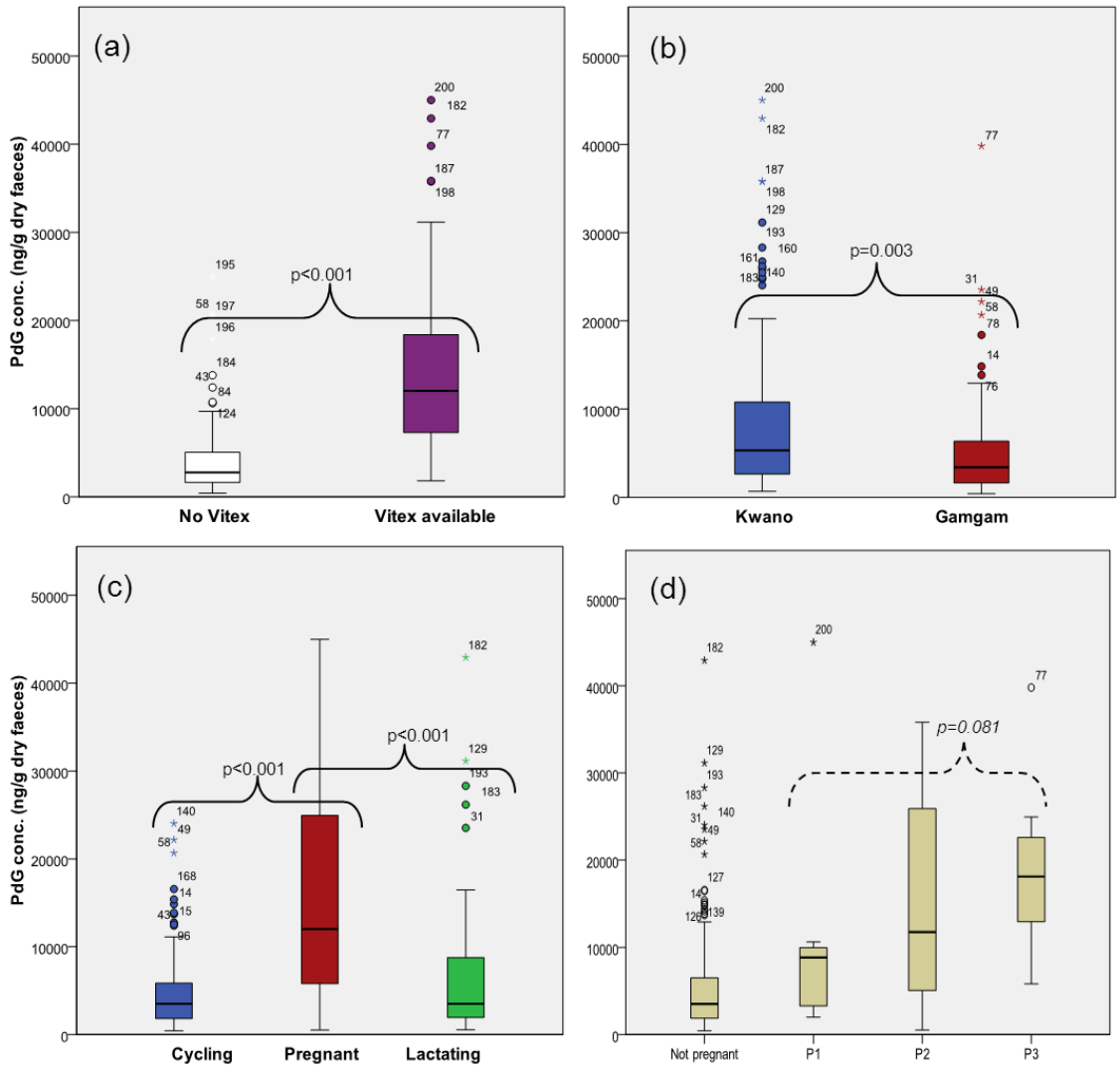

Figure 6.6. Box-plots showing variation in PdG levels (ng/g dry faeces) with (a) *V. doniana* availability, (b) troop, (c) reproductive state and (d) pregnancy stage.

Table 6.3. Variation in PdG levels (ng/g dry faeces) between pregnant and non-pregnant animals and between the three different pregnancy stages. Table shows the results of z tests from one GLMM with pregnancy stage as the single fixed variable. The coefficient represents the difference between the average PdG values for the two pregnancy stage categories.

Pregnancy stage 1	**Pregnancy stage 2**	**Coefficient**	**s.e.**	**z**	**p**
Not pregnant	P1	0.289	0.156	1.853	***0.064***
Not pregnant	P2	0.394	0.113	3.487	**<0.001**
Not pregnant	P3	0.647	0.147	4.401	**<0.001**
P1	P2	0.105	0.182	0.577	0.564
P1	P3	0.358	0.205	1.746	***0.081***
P2	P3	0.253	0.175	1.446	0.148

Interactions between *V. doniana* availability, troop and reproductive state

In order to determine whether the effect of troop was an artefact of non-random distribution of reproductive states or *V. doniana* availability between troop categories, the effect of troop was investigated while controlling for the effects of these two variables. The addition of troop to the PdG 2-factor model containing *V. doniana* availability, reproductive state and their interaction effect significantly improved the fit of the model (D=5.20, d.f.=1, p=0.023). However, there were no significant interaction effects between troop, *V. doniana* availability and reproductive state (appendix 6d, table A6.xv). For all reproductive state-*V. doniana* availability categories Kwano troop animals exhibited higher PdG levels than Gamgam troop animals. This effect was significant for pregnant animals whether or not *V. doniana* was available and marginally non-significant for lactating animals when *V. doniana* was not available (table 6.4, figure 6.7)

Table 6.4. Effect of troop on the PdG levels (ng/ g dry faeces) of faecal samples from animals in different categories of reproductive sate and *V. doniana* availability. Table shows the results of z testes from one GLMM containing troop, *V. doniana* availability and reproductive state as fixed variables. The coefficient represents the difference between the average PdG values of Kwano and Gamgam animals belonging to each of the *V. doniana* availability-reproductive state categories.

***V. doniana* availability**	**Reproductive state**	**Coefficient[1]**	**se**	**z**	**p**	**Direction of relationship**
Not available	Cycling	-0.076	0.071	1.07	0.285	Kwano > Gamgam
	Pregnant	-0.355	0.128	2.77	**0.006**	**Kwano > Gamgam**
	Lactating	-0.147	0.081	1.82	***0.070***	***Kwano > Gamgam***
Available	Cycling	-0.057	0.103	0.55	0.580	Kwano > Gamgam
	Pregnant	-0.335	0.138	2.43	**0.015**	**Kwano > Gamgam**
	Lactating	-0.127	0.098	1.30	0.195	Kwano > Gamgam

1. Difference between average Kwano and Gamgam PdG levels

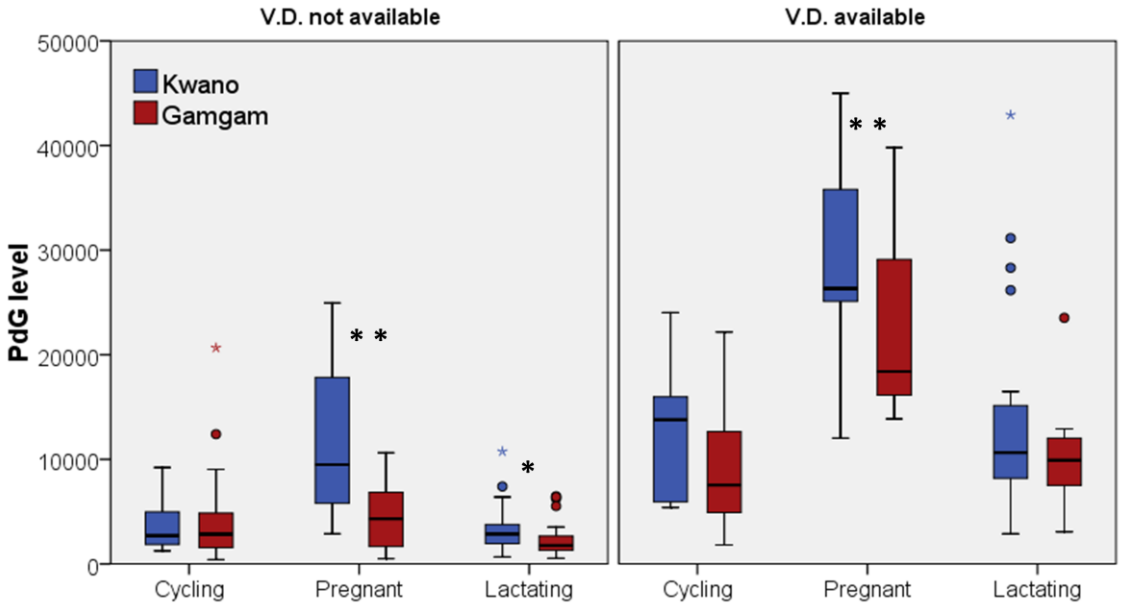

Figure 6.7. Box-plots showing the effect of *V. doniana* availability (V.D.), reproductive state and troop on PdG levels (ng/g dry faeces). Significant difference of $p<0.05$ indicated by **, difference of $p>0.05$ but <0.10 indicated by *.

This model also revealed that PdG levels were significantly higher when *V. doniana* was available compared to when it was not for all troop-reproductive state categories (table 6.5) and that pregnant animals exhibited higher PdG levels than both cycling and lactating animals in for all troop-*V. doniana* availability categories. The difference was significant in 6 and marginally non-significant in 1 out of the 8 categories (table 6.6). There was a trend for cycling animals to exhibit higher PdG levels than lactating animals but only amongst Gamgam animals when *V. doniana* was not available (table 6.6).

Table 6.5. Effect of *V. doniana* availability on the PdG levels (ng/g dry faeces) of faecal samples from animals in different categories of troop and reproductive sate. Table shows the results of z testes from one GLMM containing troop, *V. doniana* availability and reproductive state as fixed variables. The coefficient represents the difference between the average PdG values of samples collected when *V. doniana* fruit is and is not available, belonging to each of the troop-reproductive state categories.

Troop	**Reproductive state**	**Coefficient**	**se**	**z**	**p**	**Direction of relationship**
Kwano	Cycling	0.45	0.093	4.84	**<0.001**	**V.D. available > Not available**
	Pregnant	0.579	0.117	4.95	**<0.001**	**V.D. available > Not available**
	Lactating	0.645	0.077	8.38	**<0.001**	**V.D. available > Not available**
Gamgam	Cycling	0.469	0.082	5.72	**<0.001**	**V.D. available > Not available**
	Pregnant	0.599	0.130	4.61	**<0.001**	**V.D. available > Not available**
	Lactating	0.665	0.091	7.31	**<0.001**	**V.D. available > Not available**

Table 6.6. Effect of reproductive state on the PdG levels (ng/g dry faeces) of faecal samples from animals in different categories of troop and *V. doniana* availability. Table shows the results of z testes from one GLMM containing troop, *V. doniana* availability and reproductive state as fixed variables. The coefficient represents the difference between the average PdG values of cycling, pregnant and lactating animals belonging to each of the troop-*V. doniana* availability categories.

Reproductive states compared	**Troop**	**V. doniana availability**	**Coefficient**	**se**	**z**	**p**	**Direction of relationship**
Pregnant vs. Cycling	Kwano	Not available	0.405	0.100	4.05	**<0.001**	**Pregnant>Cycling**
		Available	0.534	0.126	4.24	**<0.001**	**Pregnant>Cycling**
	Gamgam	Not available	0.126	0.115	1.10	0.273	Pregnant>Cycling
		Available	0.256	0.149	1.72	***0.086***	***Pregnant>Cycling***
Pregnant vs. Lactating	Kwano	Not available	0.474	0.103	4.60	**<0.001**	**Pregnant>Lactating**
		Available	0.408	0.113	3.61	**<0.001**	**Pregnant>Lactating**
	Gamgam	Not available	0.266	0.111	2.40	**0.017**	**Pregnant>Lactating**
		Available	0.2	0.144	1.39	0.165	Pregnant>Lactating
Lactating vs. Cycling	Kwano	Not available	-0.07	0.075	0.93	0.321	Cycling>Lactating
		Available	0.126	0.101	1.25	0.212	Lactating >Cycling
	Gamgam	Not available	-0.14	0.075	1.88	***0.062***	***Cycling>Lactating***
		Available	0.056	0.105	0.53	0.594	Lactating >Cycling

6.2.3. Relationship between PdG and GC levels

The relationship between PdG and GC levels was investigated by directly comparing the levels of the two hormone metabolites from the same faecal samples using GLMMs, with faecal sample number and ID fitted as random effects. Three sets of models were built each controlling for one of the three categorical variables that were found to affect PdG level significantly (*V. doniana* availability, troop and reproductive state). These

three variables could not usually be added to the model together because the sample size within each of the categories would be too small for a meaningful analysis e.g. there was only one pregnant, Gamgam female. Full results from these three sets of models are presented in appendix 6d, table A6.xvi.

The addition of GC to the 2-factor PdG null model significantly improved its fit ($D=51.74$, d.f.=1, $p<0.001$), with a highly significant positive relationship between the two hormone levels ($z=7.61$, $p<0.001$).

The interaction between GC level and *V. doniana* availability was not significant ($D=0.23$, d.f.=1, $p=0.630$) and the significant difference between the PdG levels of samples collected when *V. doniana* was and was not available remained significant when GC level was controlled for ($z=6.78$, $p<0.001$, figure 6.8).

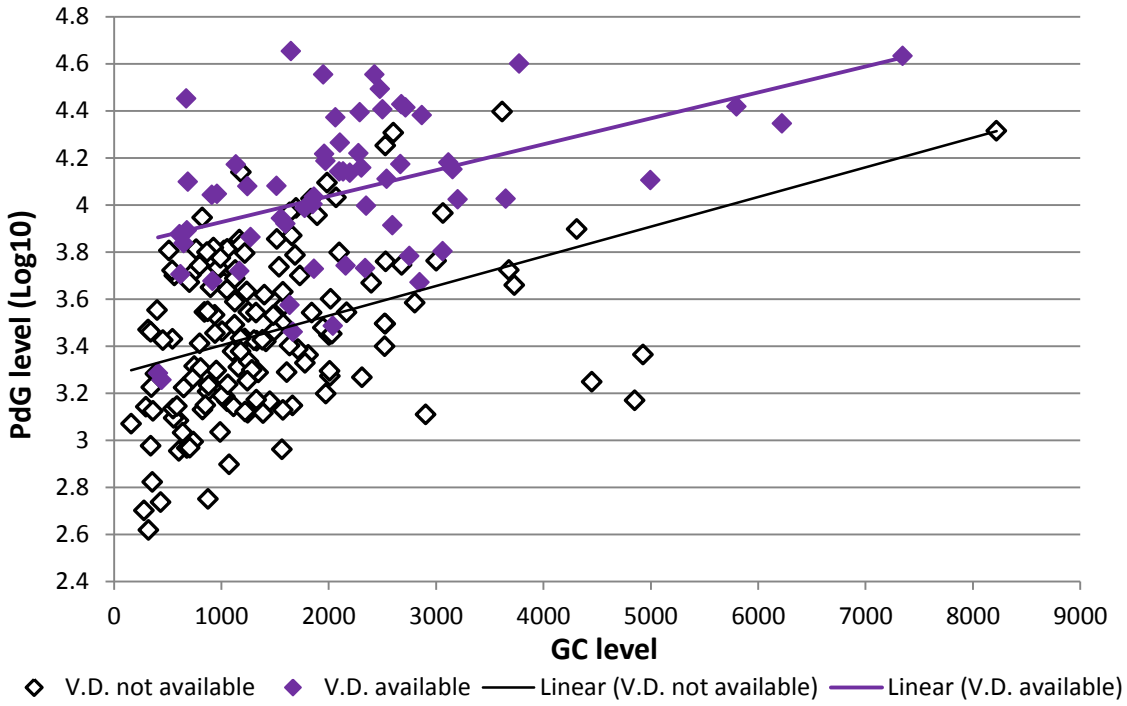

Figure 6.8. Scatter plot showing the relationship between PdG and GC levels (both in ng/g dry faeces) for samples collected when *V. doniana* (V.D.) was and was not available. The lines represent the average linear relationships between the two variables for all individuals in each V.D. availability category, as predicted by the GLMM.

The interaction between GC level and troop was not significant (D=1.28, d.f.=1, p=0.251). However, when GC level was controlled for there was no longer a significant difference between the PdG levels of Kwano and Gamgam troop members (z=1.63, p=0.103), which means that the variation previously explained by samples originating in different troops is, in the present model, explained by differences in the GC levels of samples (figure 6.9).

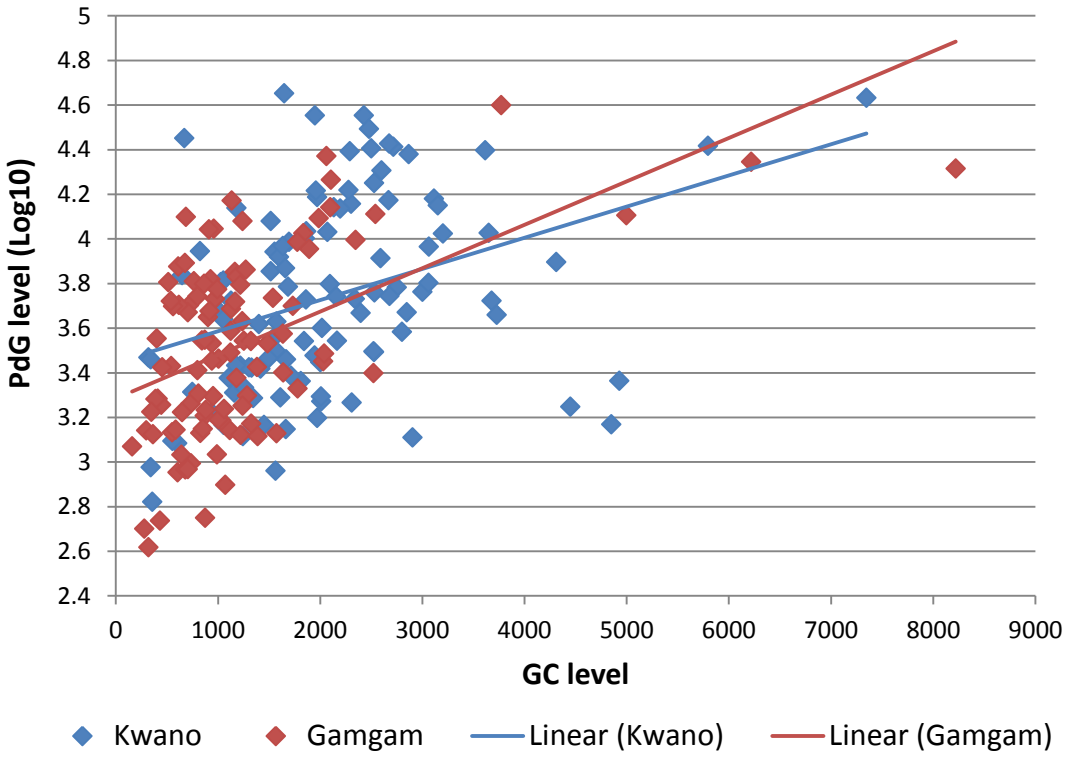

Figure 6.9. Scatter plot showing the effect of troop on the relationship between PdG and GC levels (both in ng/g dry faeces). The lines represent the average linear relationships between the two variables for all individuals in each troop, as predicted by the GLMM.

There was a significant interaction between GC level and reproductive state (D=13.93, d.f.=2, p=0.001). Although the relationship between GC and PdG level was highly significant and positive for all reproductive states (cycling: z=4.37, p<0.001; pregnant: z=5.66, p<0.001; lactating: z=6.44, p<0.001) the slopes of the lines differed, with the

steepest relationship for pregnant females, followed by lactating females and then cycling females (figure 6.10).

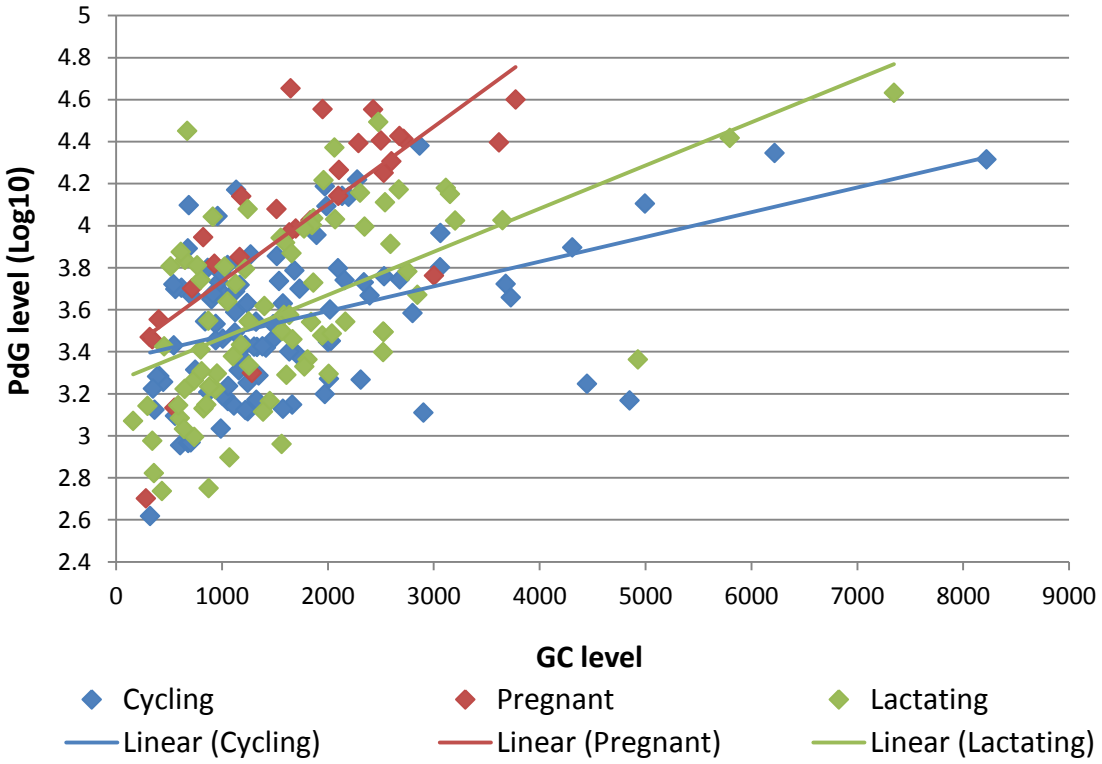

Figure 6.10. Scatter plot showing the effect of reproductive state on the relationship between PdG and GC levels (both in ng/g dry faeces). The lines represent the average linear relationships between the two variables for all individuals in each reproductive state, as predicted by the GLMM.

Relationship between PdG and GC level for luteal phase sample only

As PdG and GC levels during the luteal phase are important in influencing conception probability, the hormone levels of faecal samples collected from females in this stage of their cycle were examined separately. GLMMs were once again built, with faecal sample number (n=35) and ID of sample producer (n=7) fitted as random effects. Troop and *V. doniana* availability were included in the model as fixed effects.

The fit relative to the PdG 2-factor null model was significantly improved by the addition of GC level, troop, *V. doniana* availability and all two-way interactions

(D=22.89, d.f.=6, p=0.001). There was a significant positive relationship between PdG and GC level for Gamgam troop but not Kwano troop, whether or not *V. doniana* was available (table 6.7, figure 6.11).

The analyses were repeated without one particular data point (ID= MMK, faecal sample number=37), which had a particularly high GC value and appeared to be strongly influencing the relationship between GC and PdG value for Gamgam animals when *V. doniana* was not available, but the significance of the relationship was not affected (appendix 6d, table A6.xvii).

Table 6.7. Effect of *V. doniana* availability and troop on the relationship between PdG and GC levels (both in ng/ g dry faeces) of luteal phase faecal samples. Table shows the results of z testes from one GLMM containing GC level, *V. doniana* availability and troop as fixed variables. The coefficient represents the relationship between PdG and GC level for each *V. doniana* availability-troop category.

Category 1	**Category 2**	**Coefficient**	**s.e.**	**z**	**p**
V. doniana not available	Kwano	-0.0000768	0.0000632	1.22	0.224
	Gamgam	0.000121	0.0000313	3.87	<**0.001**
V. doniana available[1]	Gamgam	0.000612	0.000311	1.97	**0.049**

1. No luteal phase samples were collected from Kwano troop members during the period when *V. doniana* was available

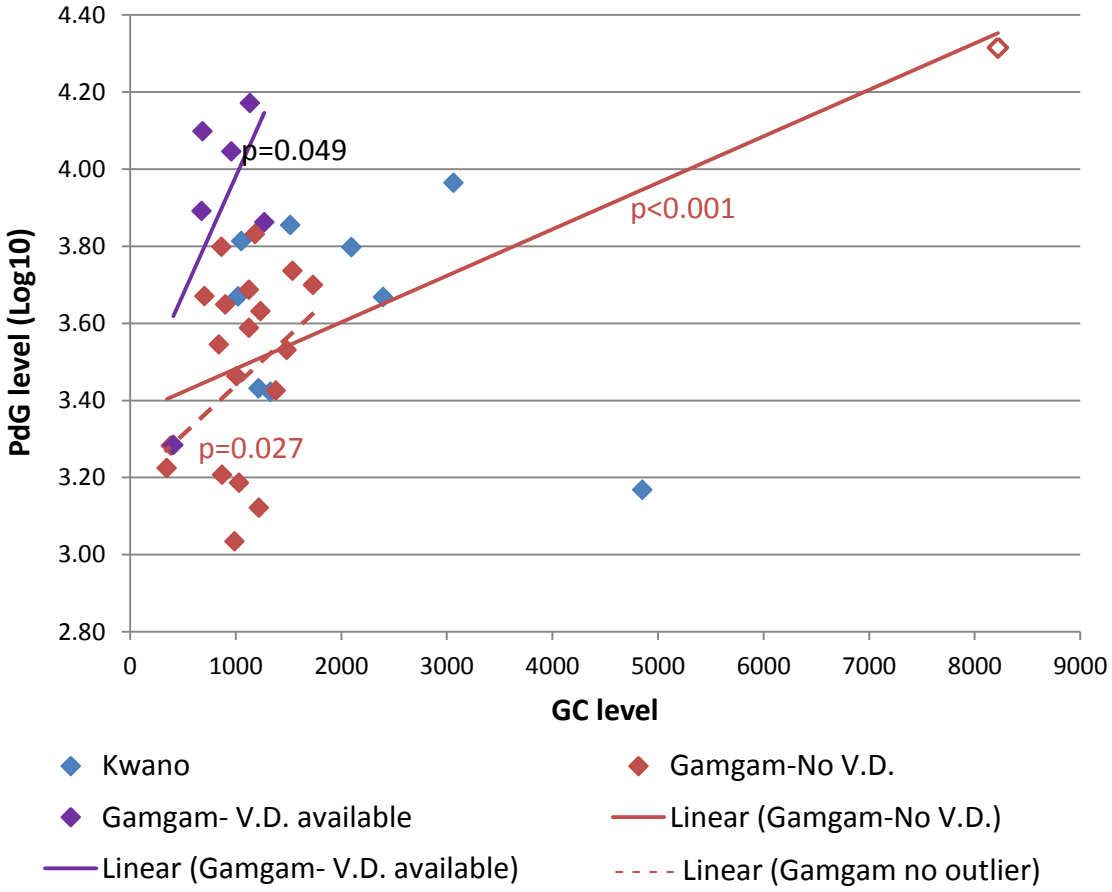

Figure 6.11. Scatter plot showing the effect of troop and *V. doniana* (V.D.) availability on the relationship between PdG and GC levels (both in ng/g dry faeces) of luteal phase faecal samples. The lines represent the average linear relationship between the two variables for all individuals in each troop-V.D. availability category. The open symbol denotes the outlier (sample number 37, from focal animal MMK) and the dashed line represents the linear relationship for this category (Gamgam troop, V.D. not available) excluding the outlier.

The possibility of cross-reactivity

It is possible that the relationships between the glucocorticoid and progesterone metabolites, shown here, are due to cross-reactivity. The two immunoassays used in the current study were both group specific assays which means that molecules of a similar structure to the assay's target molecule will be able to bind, to some extent, to the antibodies used in the assay, a property referred to as cross-reactivity. Progestogens and glucocorticoids are both classes of steroid hormones and as such have a similar molecular structure. It is therefore possible that group specific assays designed to react with either of these groups will cross-react, to some degree, with the other. If this occurs

then the observed correlation between faecal PdG and GC levels will be due, in part, to the fact that the assays are detecting the same molecules.

To the best of my knowledge, the antibody used in the GC assay (5β-androstane-3α,11β-diol-17CMO) has not been tested for cross-reactivity with the progesterone metabolite measured by the progesterone assay (pregnanediol-3-gluconide) and the antibody used in the PdG assay has not been tested for cross-reactivity with the GC metabolite measured by the GC assay (3a,11β-dihydroxy-CM). However, progesterone has been shown to have very low cross-reactivity with the GC antibody (0.015% cross-reactivity: Salimetrics, 2010) and cortisol has been shown to have very low cross-reactivity with the PdG antibody (<0.01% cross reactivity: Hodges and Green, 1989). It is therefore probable that there is no biologically significant cross-reactivity between the glucocorticoid and progesterone metabolites used in this study. The observed relationship between these two metabolites will therefore be treated as a correlation between the baboons' actual progesterone and glucocorticoid levels.

6.2.4. Relationship between PdG levels and energetic status measures

The relationships between PdG levels and the energetic status measures (calculated energy intake and expenditure rate and UCP levels) were investigated by calculating median PdG levels and energetic status measures for each individual for each month for which the appropriate data were available (n=77). GLMMs were built with individuals' median monthly PdG levels as the dependant variable, an individual-month number (1-77) and individuals' ID fitted as random effects, and one of the energetic status measures fitted as a fixed effect. Three sets of models were built for each energetic status measure, each controlling for one of the three categorical variables that were

found to significantly affect PdG level (*V. doniana* availability, troop and reproductive state). Non-significant statistical results are presented in appendix 6d, table A6.xviii-xix.

Calculated energy intake rate

When all the data were considered together there was no sign of a relationship between individuals' median monthly PdG levels and their median monthly calculated energy intake rates. The addition of *V. doniana* availability and troop to the models also resulted in no significant relationship between the variables. However, reproductive state did interact significantly with energy intake rate ($D=8.88$, d.f.=2, $p=0.012$) and revealed a significant negative relationship between individuals' median monthly PdG levels and intake rates, for lactating females only ($z=3.81$, $p<0.001$). Due to the major impact of *V. doniana* availability on PdG levels this factor was also added to these models in order to control for its effect. The new model revealed that the negative relationship between PdG levels and energy intake rates for lactating females was only present during the months when *V. doniana* was not available ($z=2.70$, $p=0.007$, figure 6.12).

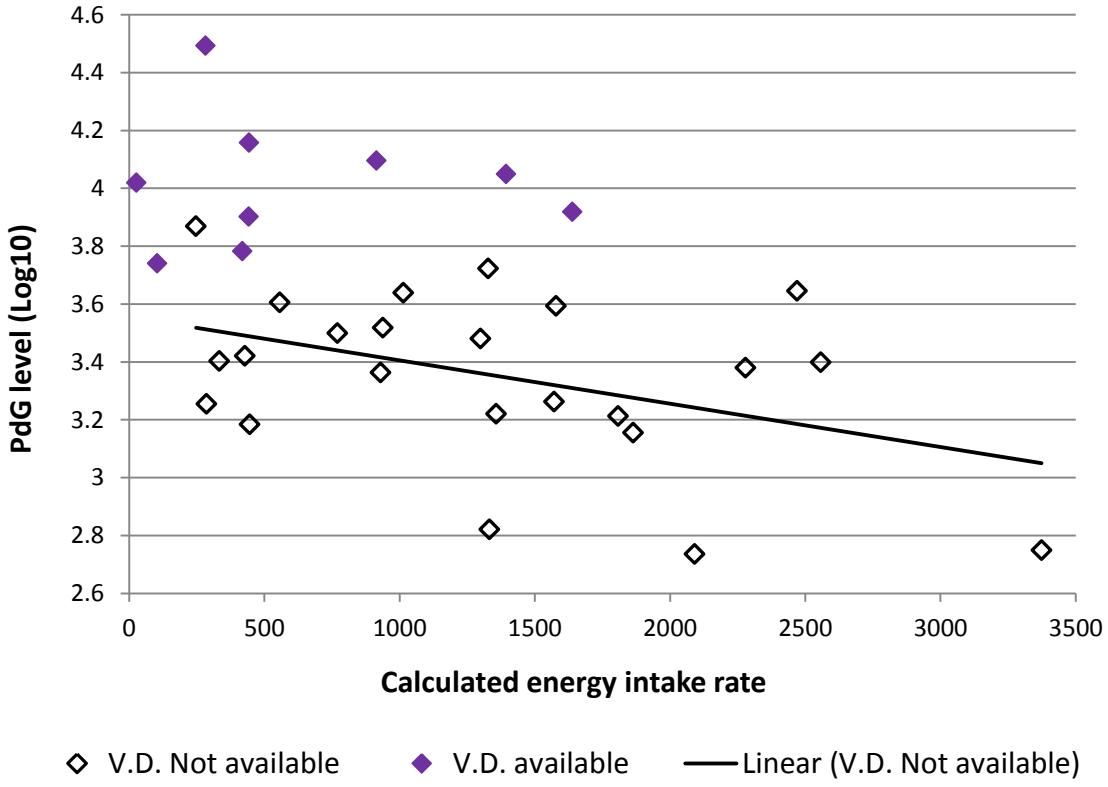

Figure 6.12. Scatter plot showing the effect of *V. doniana* (V.D.) availability on the relationship between energy intake rate (kJ/hr) and PdG level (ng/g dry faeces) for lactating animals. The line represents the average linear relationship between the two variables for all individuals when V.D. was not available.

Calculated energy expenditure rate

When all the data were considered together there was a significant negative relationship between individuals' median monthly PdG levels and energy expenditure rates (comparison with PdG 2-factor null model: D=5.38, d.f.=1, p=0.020, z=2.37, p=0.018). However, this relationship disappeared with the addition of *V. doniana* availability and its interaction with energy expenditure rate to the model (*V. doniana* available: z=0.65, p=0.518; *V. doniana* not available: z=1.02, p=0.310, figure 6.13). Similarly, a negative relationship between PdG level and energy expenditure rate present for Kwano (z=2.71, p=0.007) animals, in the model containing troop, disappeared when the *V. doniana* availability variable was added (appendix 6d).

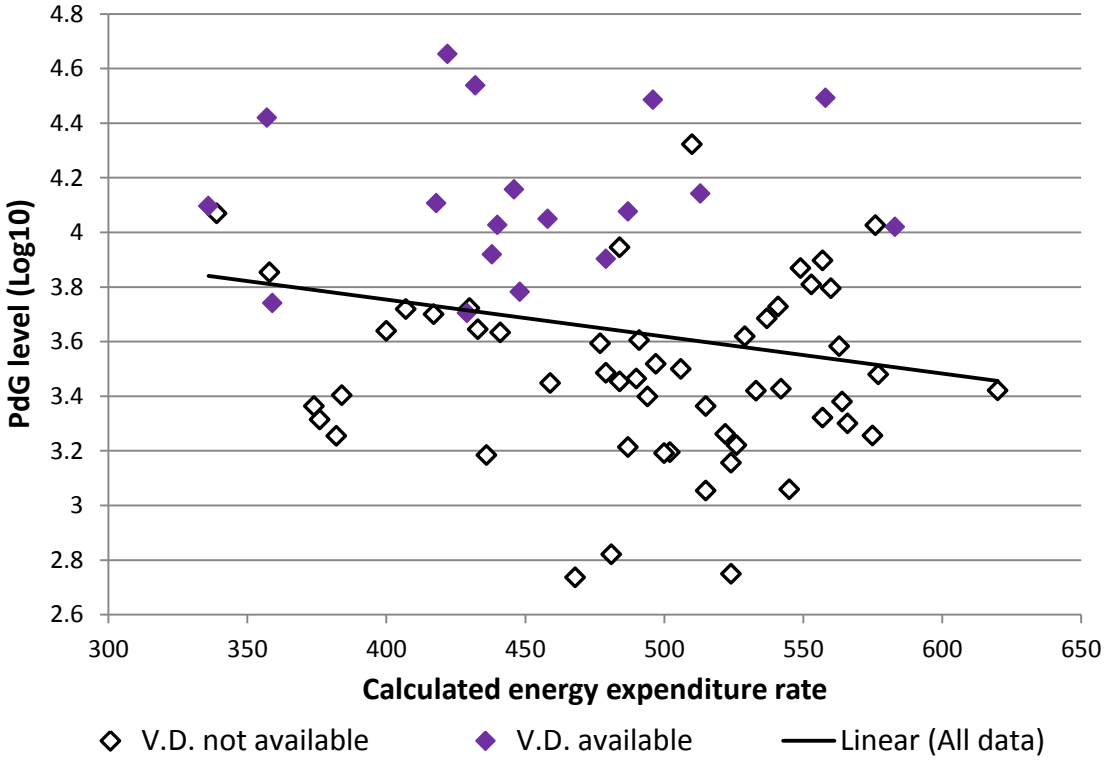

Figure 6.13. Scatter plot showing the effect of *V. doniana* (V.D.) availability on the relationship between energy expenditure rate (kJ/hr) and PdG level (ng/g dry faeces). The line represents the average linear relationship between the two variables for all individuals.

UCP levels

No evidence of a relationship between PdG and UCP level was found and UCP level did not interact significantly with either the *V. doniana* availability, troop or reproductive state categories (see appendix 6d, table A6.xix for results).

6.2.5. Influences on individual reproductive rates

A value of the rate of production of yearling offspring (infants surviving to 12 months) was calculated by dividing the number of offspring that each focal animal had produced that had survived to one year by the total number of months each animal has spent as a mature adult over the ten year period since the troops were first habituated (Warren, 2003). Percentage infant mortality was also calculated for each female using the

following equation: % infant mortality = (no. infants surviving to 1 year / total no. infants) x 100 (table 6.8).

Table 6.8. Yearling production rates and % infant mortality for adult females from both troops.

ID	Observation months[1]	No. Infants born within obs. months	No. infants died aged < 1 year	No. yearlings produced[2]	Yearling production rate	% infant mortality
BRA	115	4	2	2	0.017	50
DRK	52	2	0	2	0.038	0
FDI	63	2	0	2	0.032	0
KYE	71	2	0	2	0.028	0
LDI	111	4	0	4	0.036	0
LMI	120	5	3	2	0.017	60
MOM	61	4	2	1	0.016	50
SDY	71	2	0	2	0.028	0
TJL	116	3	0	3	0.026	0
YMK	86	3	0	2	0.023	0
Kwano total	**866**	**31**	**7**	**22**	**0.025[3]**	**21.88[3]**
BUD	115	4	0	3	0.026	0
KAN	68	3	0	3	0.044	0
MMK	115	4	2	2	0.017	50
MMW	115	7	1	5	0.043	14
STR	115	6	1	4	0.035	17
Gamgam total	**528**	**24**	**4**	**17**	**0.032[3]**	**16.67[3]**
Both troops total	**1394**	**56**	**11**	**39**	**0.028[3]**	**19.64[3]**

1. Number of months between the time the animal was either first identified and named (as an adult) or the time when a female previously identified as a sub-adult reached adult status due to the observation of her first pregnancy, and the most recent record of that animal's reproductive status (Dec 2010 for Gamgam animals and May 2011 for all Kwano animals except KRM who disappeared Jan 2010).
2. At the time of data collection some of the infants mentioned had not yet reached 1 year which is why the number of yearlings produced is less than the number of infants born minus the number of infants that died aged less than 12 months.
3. Total values for yearling production rate and % infant mortality were calculated using summed values from all individuals/ all individuals in a troop rather than by calculating average values e.g. total % infant mortality for Kwano troop = total no. infants born (31) / total no. infants died (7) * 100 = 21.88.

Effect of troop on yearling production rate and offspring mortality

Gamgam troop had a higher yearling production rate than Kwano troop but the difference was not significant (independent samples t-test, t=-1.389, d.f=13, p=0.188, figure 6.14).

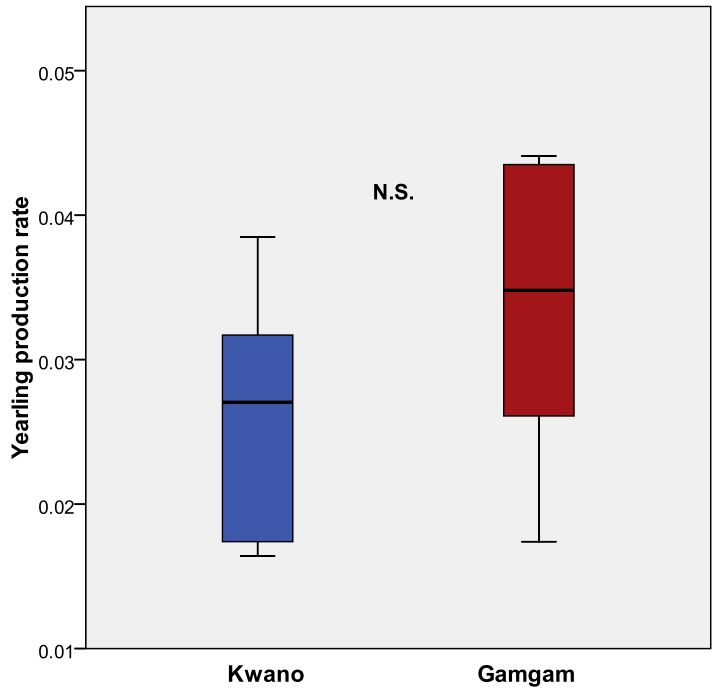

Figure 6.14. Box plot showing the effect of troop on yearling production rates.

Total percentage infant mortality was higher for Kwano troop than for Gamgam troop (table 6.8, figure 6.23) but there was no significant difference between the two troops in terms of the infant mortality rates for individual animals (Mann Whitney U test: U= 21, n=15, p=0.579).

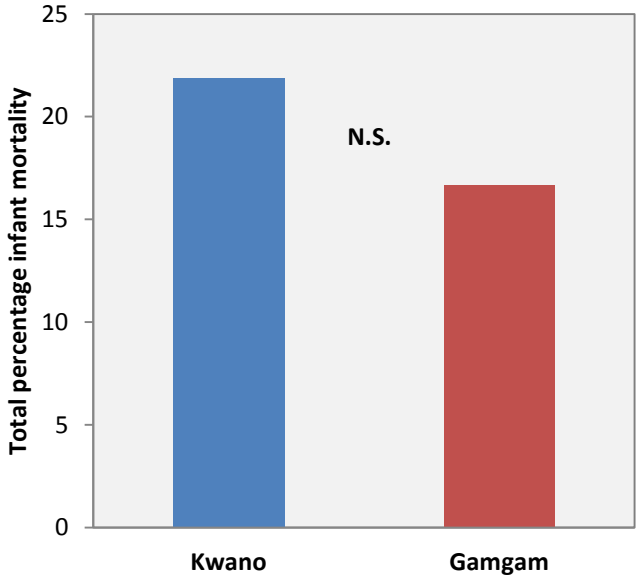

Figure 6.15. Bar chart showing total percentage infant (<1 year) mortality for Kwano and Gamgam troops.

Relationship between yearling production rate and the hormone and energetic status measures

The relationship of yearling production rate with the hormone and energetic status variables was assessed using GLMs with ID as the single random factor, infant survival rate as the dependent variable and one of the following factors entered into the model as a fixed factor: PdG, GC and UCP level, calculated energy intake rate and expenditure rate. The variable troop and its interaction effect were included, as fixed factors, in each of these models in order to determine whether the relationships differed between the two troops. The effect of rank on yearling production rate and the effect of rank on the relationship between yearling production rate and the other variables was also tested but no significant effect was detected so results are not presented here (appendix 6d, table A6.xxi).

Yearling production rate was highly significantly related to calculated energy intake rate within both troops (comparison with null model: $D=21.89$, d.f.=3, $p<0.001$). The direction of this relationship was positive for Gamgam troop ($z=5.00$, $p<0.001$) but negative for Kwano troop ($z=4.02$, $p<0.001$) (figure 6.16).

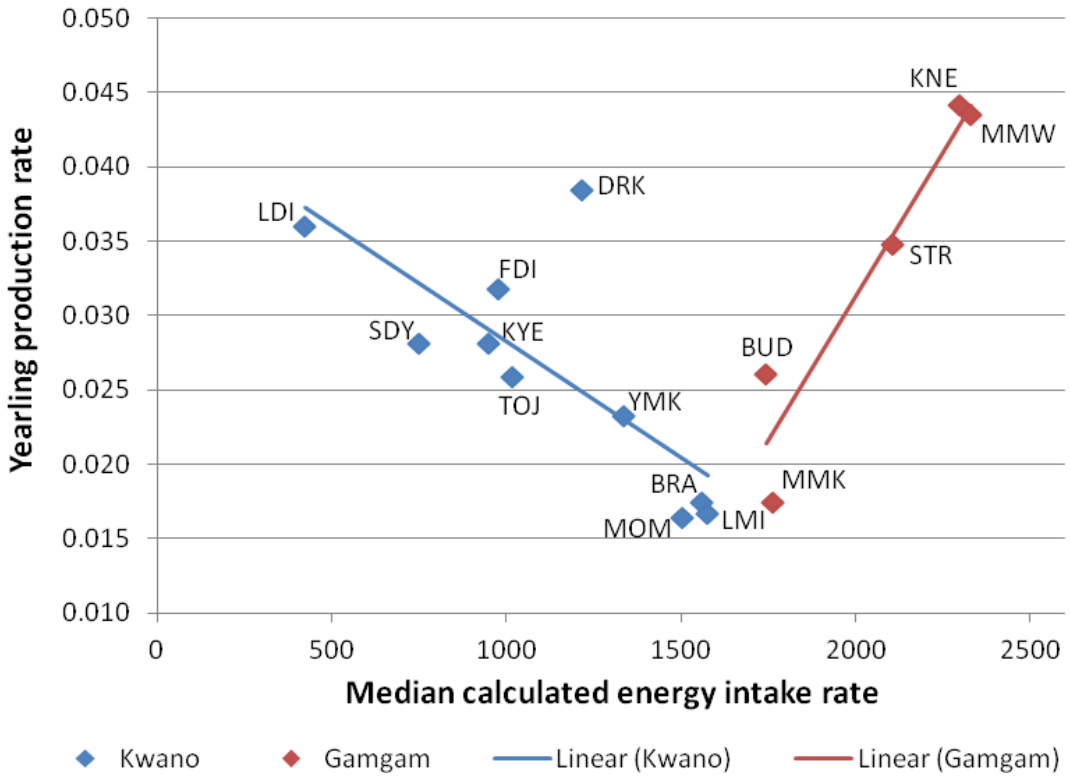

Figure 6.16. Scatter plot showing the effect of troop on the relationship between individuals' yearling production rate and their median calculated energy intake rate. The lines represent the linear relationship between the two variables for each troop.

Yearling production rate was not significantly related to GC level, UCP level or energy expenditure rate for either troop (appendix 6d, table A6.xxi). There was a marginally non-significant positive relationship between individuals' median PdG levels and their yearling production rates (comparison with null model: D=6.49, d.f.=3, p=0.090) for Kwano troop (z=1.75, p=0.080), although this relationship appears to be driven largely by the results of one female, DRK and the removal of this data point results in the relationship becoming non-significant (z=0.39, p=0.694) (figure 6.17).

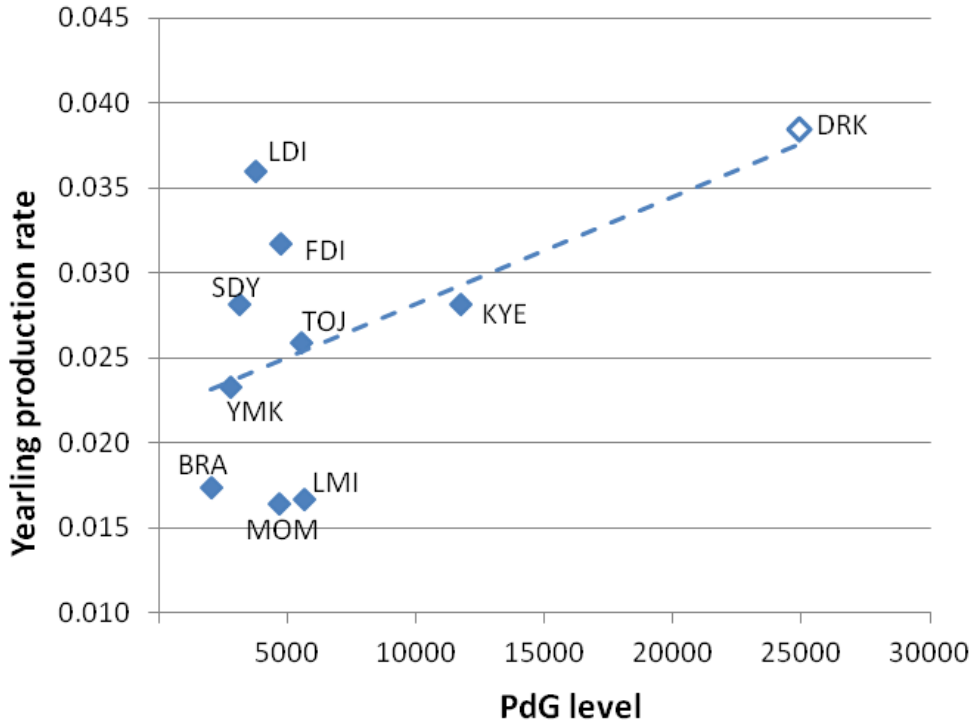

Figure 6.17. Scatter plot showing the relationship between Kwano troop members' yearling production rate and their median PdG levels (ng/g dry faeces). The open symbol denotes an outlier and the dashed line represents the linear relationship between the two variables when the outlier is included.

6.3. Discussion

6.3.1. Effect of *Vitex doniana* fruit availability and reproductive state on PdG levels

As discussed in section 6.1.2 previous studies of the Gashaka baboons identified the fruit and new leaves of *V. doniana* as an external source of progesterone like compounds, with consumption of these items resulting in seasonal progesterone peaks (Higham *et al.*, 2007). The results of the present study mirror these results by demonstrating elevated PdG levels in faecal samples from females of all reproductive states collected during the period that *V. doniana* is known to occur and was observed to be eaten by the baboons. It is difficult to draw conclusions on whether this exogenous source of progesterone inhibited the reproduction of the Gashaka baboons during the study period. None of the six conceptions which took place during the study period occurred within the months of presumed *V. doniana* fruit consumption (August-September), during which time one Kwano focal animal (LMI) and three Gamgam focal

animals (BUD, MMK, STR) were cycling. This is consistent with the previous study, which also recorded no conceptions during the period of *V. doniana* fruit consumption (Higham *et al.,* 2007). However, sexual swellings were observed during the period in all three cycling Gamgam animals and from a subadult Kwano female (RAB, who was not one of the study's focal animals) although not for the single, cycling Kwano focal animal.

Consistent with normal patterns of primate progesterone production, the PdG levels of samples from pregnant females were higher than those from cycling and lactating females. The lack of a significant interaction effect between reproductive state and *V. doniana* availability means that the elevation in PdG levels evident during August and September occurred in animals irrespective of their reproductive state providing further support for the hypothesis that an external source (i.e. *V. doniana* fruit) is responsible for the unusually high PdG levels observed during this period.

6.3.2. Effect of troop on PdG levels

Troop had a significant effect on PdG level which remained when the effect of reproductive state and *V. doniana* availability were controlled for and did not differ significantly between animals in different reproductive states or between time periods when *V. doniana* was and was not available. As predicted, Kwano troop exhibited consistently higher PdG levels than Gamgam troop, consistent with the result from the 2004-5 Gashaka study (Higham, 2006). Higham (2006) suggested that this difference could be due to the fact that Kwano troop had lower nutritional status than Gamgam troop, a factor which has been found to result in relatively higher PdG excretion in red deer (Cook *et al.,* 2001). This idea is consistent with the activity budget and calculated energy measure results from chapter 3, which showed that Gamgam troop spent less

time in energetically expensive behaviors and exhibited substantially higher energy intake rates compared to Kwano troop. The difference between the two troops may also be related to adrenal production of progesterone due to stress (section 6.1.1) which will be discussed further below.

6.3.3. Relationship between progesterone stress and energetics

There was a highly significant positive relationship between PdG and GC levels when all the data were considered together, whether or not *V. doniana* was available and within both troops. Within all three reproductive states the relationship between these two variables was also highly significant and positive but the slopes of the relationship differed significantly between the categories, with the steepest slope for pregnant females' samples and the least steep slope for cycling females' samples. When only samples from the luteal phase, i.e. the period when PdG level can influence implantation success, were considered there was a significant positive relationship for Gamgam troop only, whether or not *V. doniana* was available. The lack of a relationship for Kwano troop may be related to the small number of luteal phase samples collected from this troop (Kwano, n=9; Gamgam, n=26).

The positive relationship between PdG and GC levels is as predicted and consistent with the idea that stress increases progesterone production, most likely via the adrenal gland (Fajer *et al.*, 1971; Plotka *et al.*, 1983; Cook, *et al.*, 2001). This result is also consistent with the fact that both the PdG (section 6.4.3) and GC (chapter 5) levels exhibited by Kwano troop animals were significantly higher than those exhibited by Gamgam troop animals. However, this result is also in contrast to several studies of non-human primates showing reductions in progesterone levels due to stress (Guinea baboon: Albrecht *et al.*, 1979; rhesus macaque: Hayashi and Moberg, 1990; Xiao *et al.*, 1999;

Xiao *et al.,* 2002; long-tailed macaque: Williams *et al.,* 2007). For cycling females the increase in progesterone production, due to stress, is thought to be adaptive since it acts to inhibit reproduction during times of hardship (Plotka *et al.,* 1983). This idea is consistent with the results of Wasser's (1996) study showing that the progesterone levels of baboon conceptive cycles were highest during times of stress and also consistent with his idea that progesterone thresholds for successful implantation increase during times of stress in order to reduce the chances of becoming pregnant under these conditions. For the Gashaka baboons the significant positive relationship between the PdG and GC levels of cycling females suggests that this process may be acting, but due to the small total number of conceptions that occurred during the study period (5 for Kwano, 1 for Gamgam) it is not possible to draw meaningful conclusions about the effect of this variation in PdG levels on conception success. The significant relationship between PdG and GC levels found during pregnancy is also consistent with the ideas of Plotka *et al.* (1983), in particular that elevated progesterone levels during pregnancy help to prevent spontaneous abortion than can be triggered by high GC levels. Another interesting result to come out of the PdG-GC correlations is that when GC level was controlled for there was no longer any significant difference between the PdG levels of the two troops. This result suggests that the difference between the progesterone levels of the two troops may well be driven by higher stress levels experienced by Kwano troop members relative to Gamgam troop members.

No significant effect of either calculated energy expenditure or UCP level on PdG level was found. However, calculated energy intake rate was significantly and negatively related to PdG level but only for lactating animals when *V. doniana* was not available. Lactation represents a transitional period during which, as infants get older and suckling decreases, the chances of a female resuming cycling and becoming able to conceive

increase. In humans, this transition is known to be mediated by a female's energetic status, with a relatively high energetic status necessary for resumption of cycling (Ellison, 1990; Ellison, 2003). Since high progesterone levels can inhibit ovulation and fertilisation it makes sense that lactating animals under energetic stress exhibit these high PdG levels which may inhibit the initiation of follicular maturation and therefore reproduction during the unfavourable conditions (Plotka *et al.,* 1983; Wasser, 1996).

As discussed in section 6.1.1.2, there are several possible explanations for a negative association between energy intake and progesterone level including the association between increased stress and increased adrenal progesterone production and the elevation of metabolic clearance rates of progesterone associated with consuming a large mass of food. It seems unlikely that free-ranging primates could achieve food intake of a mass comparable to that fed to domestic livestock unless there were particularly high levels of food-enhancement, which makes the metabolic clearance hypothesis (e.g. Parr *et al.,* 1987) seem less likely. In contrast the stress hypothesis seems more plausible and is also supported by the strong positive association found between the baboons' progesterone and glucocorticoid levels.

The weak evidence for a link between the Gashaka baboons' progesterone levels and their energetic status measures is in contrast to the significant relationships presented in previous chapters between the energetic status measures and other variables (Chapters 2-5). These included significant positive relationships between calculated energy intake rate and the fruit indices, between UCP level and rainfall and between calculated energy expenditure rate and GC level, as well as significant negative relationships between calculated energy expenditure rate and rainfall, calculated energy expenditure rate and UCP level and between calculated energy intake rate and GC level. It seems likely that

the lack of consistent relationship between progesterone levels and the energetic status measures is because progesterone may correlate negatively (e.g. Cook *et al.*, 2011) or positively (e.g. Emery Thompson *et al.*, 2007) with energy intake. Hence the 'optimum' progesterone level for reproductive success, and therefore the level we would expect to see associated with optimum energetic conditions, depends on reproductive state and cycling stage e.g. high progesterone levels inhibit follicular development but promote implantation (Norris, 2007).

6.3.4. Influences on yearling production rate and infant mortality

Effect of troop on yearling production rate and infant mortality

Between 2000 and 2010 Gamgam troop did have, on average, higher yearling production rates and lower infant mortality than Kwano troop. However, these differences were not statistically significant. This lack of significance is not as predicted given the energetic benefits associated with Gamgam troop's crop-raiding behaviour (Chapter 3). The result is also in contrast to a previous analysis of the baboons' reproductive performance, which found significantly shorter inter-birth intervals and substantially lower infant mortality for Gamgam troop compared to Kwano troop (Higham *et al.*, 2009). The previous analysis was based on around four and a half years of data on 10 adult females whereas the current study uses up to 10 years of data on 16 adult females. The current results suggest that the apparently substantial difference in reproductive success previously assigned to the two troops is not so pronounced when a longer period is examined. The small differences between Kwano and Gamgam troops' reproductive parameters are also in contrast to the results of other studies comparing wild-feeding and food-enhanced primates, which have found substantial increases in various measures of reproductive success associated with food-enhancement (e.g. Altmann and Alberts, 2003b). These results are, however, consistent with the idea that

the benefits, and therefore likely reproductive effects, of a relatively subtle form of food-enhancement, such as crop-raiding, are expected to be less than other forms of food-enhancement such as refuse raiding and deliberate provisioning (section 1.2.2; Higham *et al.,* 2009) and are therefore consistent with the finding that the activity budgets of the two troops differed less than those of other wild-feeding vs. food-enhanced troops from other populations (chapter 3).

Correlates of yearling production rate

Yearling production rate did not correlate significantly with individuals' median PdG, GC or UCP level or energy expenditure rate, whether the data from the two troops were considered together or separately. However, there was a significant relationship between yearling production rate and energy intake rate, for both troops. For Gamgam troop, this relationship was positive, which is as expected as females with higher energy intake are likely to be in a better position to produce and care for offspring and indeed increases in food availability or intake have been associated with increases in reproductive success in several non-human primate species (Mori *et al.*, 1997; Bentley, 1999; Altmann and Alberts, 2003b; Emery Thompson *et al.,* 2007; Garcia *et al.,* 2009). In contrast, the relationship between yearling production rate and energy intake rate was negative for Kwano troop, which is the opposite of what was predicted. This result is surprising given the well established link between positive energetic status and reproductive success (see section 6.1.4 for references). A possible explanation is that the high energy intake rates observed alongside reduced yearling production may be the result of a greater disease or parasite burden causing increased nutritional demands alongside reduced body condition and, perhaps, increased infant mortality risk (Chapman *et al.,* 2006).

6.4. Summary of results in relation to the original hypotheses

Hypothesis 1: The crop-raiding behaviour of Gamgam troop provides it with energetic benefits.

- Kwano troop exhibited significantly higher progesterone levels than Gamgam troop and evidence is presented which links this difference to the relatively high glucocorticoid levels of Kwano troop members.
- Gamgam troop exhibited higher birth and yearling production rates and lower infant mortality than Kwano troop but the differences were not statistically significant.

Hypothesis 2: Within troops, the baboons' condition will vary according to reproductive state and rank and the various measures of condition will co-vary.

- Pregnant animals exhibited significantly higher progesterone levels than cycling and lactating animals, consistent with normal patterns of primate progesterone production.
- No evidence for an effect of rank on progesterone levels or reproductive rate was found.
- A strong positive association between progesterone and glucocorticoid levels, which overrides the effect of troop, was revealed.
- Some evidence of a negative association between progesterone levels and energy intake is presented.
- Contrasting relationships between energy intake and yearling production rate are presented for the two troops: a positive correlation for Gamgam troop but a negative correlation for Kwano troop
- Neither calculated energy expenditure nor UCP level were significantly related to PdG level or the reproductive rates.

Chapter 7

CONCLUSIONS

This thesis has investigated how baboons' energetic status and condition varies over time (i.e. nine months), as weather conditions and food availability vary, and between individuals, with different energetic costs and differential access to resources. In this final chapter a summary and synthesis of the project's findings is presented, followed by a discussion of the relevance and implications of the findings. The limitations of the methods used and possible avenues for further work relating to this project are also discussed.

7.1. Summary and synthesis of findings

7.1.1. Variation between troops

Previous research identified substantial differences between the activity budgets and life-histories of Kwano and Gamgam troops, differences attributed to the energetic benefits of Gamgam troop's crop-raiding behaviour (Warren *et al.*, 2011). The current study has revealed additional differences between the two troops, which provide extra support for this finding. Consistent with previous results, this study demonstrated a difference in activity budgets between the two troops, with Gamgam troop spending more time resting and less time feeding than Kwano troop (chapter 3). Despite the relatively reduced feeding time, Gamgam troop spent significantly more days in energy surplus and also achieved substantially higher energy intake per hour than Kwano troop, which is a least partly due to the greater energetic quality of crop foods (chapter 3). The current study also revealed another possible benefit of crop-raiding in the form of relatively lower stress levels (chapter 5), a result which suggests that the reduction in

energetic stress due to crop-raiding outweighs any possible increases in psychosocial stress due to increased conflict with humans.

As well as the simple troop differences in condition measures, the relationships between the various condition measures and environmental variables (weather and fruit indices) also differed between the two troops. In most cases the relationships between these variables were significant for Kwano troop but not for Gamgam troop (e.g. negative relationships: energy expenditure rate vs. UCP level, vine & tree-fruit index vs. GC level, energy intake rate vs. GC level; positive relationship: energy expenditure rate vs. monthly maximum temperature; higher UCP level during the wet season compared to the dry season). These findings suggest that the condition of Kwano troop members is more heavily influenced by environmental factors than that of Gamgam troop members and provides support for the idea that food-enhancement buffers an animal's condition against environmental factors (Bronikowski and Altmann, 1996) and energetic stressors (Muller and Wrangham, 2004).

Previous research, based on data collected between 2000 and 2006, identified substantial differences between the two troops' reproductive parameters. Gamgam troop exhibited far lower infant mortality than Kwano troop as well as substantially shorter average inter-birth intervals (calculated from average durations of post-partum amenorrhea, cycling to conception, and gestation length), mainly due to significantly shorter durations of cycling to conception (Higham *et al.,* 2009a). The current study used different methods for quantifying reproductive performance, as well as a longer dataset, and although no significant differences were found between the two troops' birth, yearling production and infant mortality rates in each case there was a trend for reproductive performance to be better in Gamgam troop than Kwano troop. Although

the lack of a significant difference may be due to the simpler analyses employed for the current study it may also be related to the fact that a longer dataset was used. For example, infant mortality between 2000 and 2006 stood at around 45% for Kwano troop and around 6% for Gamgam troop whereas the current dataset, with an extra 3-4 years of data, finds mortality at around 25% for Kwano and 16% for Gamgam troop. However, this time scale (c. 10 years) is still small on an evolutionary scale and compared to other long-term primate study sites (Kappeler and Watts, 2012). Changes in group size over time (e.g. Gamgam troop increased from 14-20 group members between 2000 and 2009) could also have had an impact on the reproductive performance of the troops. Despite these caveats, this finding suggests that the differences between the reproductive performances of the two troops may not be as great as previously assumed, which is consistent with the idea that crop-raiding represents a relatively minor form of food-enhancement (Higham *et al.*, 2009a).

In a broader context, the apparent benefits of Gamgam troop's crop-raiding behaviour, identified by the current study, are consistent with a multitude of previous studies (section 1.2) that have demonstrated links between energy balance and fitness, or, more commonly, between proxies for energy balance (e.g. food intake, energy expenditure, habitat quality, environmental conditions) and proxies for fitness (e.g. current physical fitness, body condition, reproductive performance).

7.1.2. Variation between reproductive states

Due to the costs inherent in pregnancy and lactation, animals in these reproductive states were expected either to alter their energy intake and/or expenditure rates relative to cycling animals or to suffer energetic consequences which, it was predicted, would emerge as reduced UCP levels and/or elevated GC levels. Pregnant animals exhibited

lower energy expenditure rates as well as signs of increased intake rates, relative to cycling animals, but their UCP and GC levels did not differ from those of cycling animals. These results suggest that pregnant baboons at Gashaka are able to successfully offset their extra energetic costs by altering their energetic intake and expenditure. In contrast, lactating animals decreased their energy expenditure to a lesser extent than pregnant animals and did not increase their energy intake, exhibiting significantly lower levels than pregnant animals. Despite this, the UCP and GC levels of lactating animals provided no evidence of energetic stress although the possibility of weight loss during lactation cannot be ruled out.

Given that lactating animals have elevated energetic demands (National Research Council, 2003), decreased foraging efficiency (Silk, 1987) and an increased need for vigilance (due to the vulnerability of their infant to predation and infanticide) (Barrett *et al.*, 2006), the following scenario has been proposed for energetic management, with adjustments based on the availability and quality of food in the habitat (Barrett *et al.*, 2006). Lactating animals will reduce their activity levels, relative to cycling levels, limiting their own energy demands to compensate for those of their infant, rather than increasing feeding time (since foraging efficiency is decreased and the need for vigilance, which is positively associated with resting behaviours but negatively associated with active behaviours, is increased) (Barrett *et al.*, 2006). However, the reduction in activity levels will be limited by the need for behavioural synchrony between group members (King and Cowlishaw, 2009), which means that energy expenditure may not be sufficiently reduced to fully compensate for the infant's energetic demands. In this situation a certain degree of weight loss may be tolerated and the level of tolerance will depend on the size of fat reserves built up prior to and during pregnancy, which will in turn depend on food availability during that period. If weight

loss levels become, or are predicted to become, unacceptable, feeding time will be increased, which appears only to be necessary in low quality habitats (Barrett *et al*. 2006). For the lactating Gashaka baboons, the lack of energy intake rate adjustment together with the, most likely, negligible expenditure rate reduction may be related to the relatively high quality habitat. It may be that, through increased energy intake rates, females are able to build up sufficient fat reserves during pregnancy to see them through the energetically costly period of lactation, without having to attempt to increase energy intake during lactation, at a time when foraging efficiency is reduced and increased vigilance is necessary. If this is the case, we would expect the Gashaka baboons to lose weight during lactation. The long inter-birth intervals of Kwano troop, relative to other baboon populations (Higham *et al.*, 2009a), may be indicative of weight loss during lactation and the need to lay down energy reserves before the next reproductive attempt. The fact that elevated UCP levels were not found during lactation seems to contradict this lactational weight loss scenario but may be due to the lumping together of samples from females in all stages of lactation, since, at least in humans, insulin levels tend to be low at the beginning of lactation but rise to above normal cycling levels prior to the resumption of cycling (Valeggia and Ellison, 2009).

In general, the relationships between the condition measures and the external variables differed little between Gashaka females in different reproductive states. However, positive relationships between GC levels and both vine-fruit index and energy intake rates were significant for pregnant and lactating animals but not for cycling animals. This may suggest that during energetically expensive life history stages (i.e. pregnancy and lactation), the impact of energetic stressors is greater than at less energetically costly stages. This effect mirrors the buffering effect of food-enhancement against the influence of energetic stressors on the Gamgam baboons' condition: cycling animals

appear buffered against the effect of external energetic stressors, which have a significant impact during energetically expensive periods, by their relatively low energy intake requirements.

These findings highlight the fact that variation in nutritional requirements between individuals or across time does not necessarily translate into easily predictable changes in behaviour. As discussed in section 1.1, many factors influence the diets of individual primates and an individual's energetic requirements must be realised in concert with a suite of other requirements and constraints (National Research Council, 2003; Barrett *et al.*, 2006).

7.1.3. Variation between ranks

A female's position in a social hierarchy has often been found to be an important determinant of condition and reproductive success in cercopithecine monkeys, with variation in foraging behaviour proposed as a driver of this effect (see section 1.2.3. for references). The current study has found some evidence of a rank effect for the Gashaka baboons, with high ranking animals from Kwano troop demonstrating a trend for reduced travel time, and amongst pregnant animals only, significantly elevated energy intake rates relative to middle and low ranking animals. In contrast, low ranking animals were found to exhibit significantly higher UCP levels than middle and high ranking animals, perhaps due to an increased parasite burden amongst higher ranking animals (section 4.3.5).

In general, little consistent effect of rank was found on the Gashaka baboons' activity budgets and energy intake and expenditure rates and no effect was found on the baboons' glucocorticoid levels, progesterone levels or reproductive output. Previous

studies of the Gashaka baboons have failed to obtain a significant linear hierarchy amongst the troops' adult females (e.g. Higham *et al.,* 2009b) and although the hierarchies created for the current study were significantly linear for both troops, for Kwano troop the hierarchy was not strongly linear (Vervaecke *et al.,* 2000) and for Gamgam troop the directional consistency index of the hierarchy was very low. These findings together suggest that the dominance hierarchy amongst the female baboons at Gashaka is not particularly strong and appears to have little influence on the fitness of individual females. This may well be due to the high productivity of the Gashaka habitat, especially in relation to other baboon study sites where substantial rank effects have been found (e.g. Altmann and Alberts, 2005). If food is generally more abundant, and not particularly clumped, food competition and therefore the importance of rank may be reduced (Barton *et al.,* 1996). This idea appears to be supported by the fact that the two instances where an apparent benefit of high rank was detected in this study, involved animals under relatively greater energetic stress (decreased travel time for high ranking animals from Kwano but not Gamgam troop; increased energy intake rates for pregnant but not cycling animals). Inter-group competition, and therefore the importance of rank, may also be reduced by the generally small troop sizes in this population (Ross *et al.,* 2011) and by the ability of troops to split into even smaller groups during foraging, which has been observed at Gashaka, especially for Kwano troop (N. Alberts pers. comm.). This type of behaviour may help to ameliorate some of the costs of low rank, such as exclusion from high quality resources and receipt of aggression, as lower ranking animals could forage in a different location from higher ranking animals that might otherwise disturb them. Small troop size and troop-fission may be made possible at Gashaka by the fact that predation risk and perceived predation risk in this habitat are likely to be low, relative to the risks experienced by savannah living baboons in Eastern and Southern Africa (Ross *et al.,* 2011), due both to the

generally low predator densities of West Africa (Kunz and Linsenmair, 2007) and the high number of refuges provided by the forested environment (Cowlishaw, 2007).

7.1.4. Variation in condition measures over time

By spanning a period of ten months, this project has allowed the influences of environmental variables on baboon behaviour and condition to be investigated. Previous research has highlighted how the hottest and driest months, in arid regions, and the months with the coldest or shortest days, in temperate regions, act as environmental stressors to animals, being associated with reductions in body condition and increases in stress levels (e.g. Saltz and White, 1991; Cavigelli, 1999; Weingrill *et al.*, 2004; Gesquire *et al.*, 2008). Environmental stressors can act via their influence on time budgets, thermoregulation, water availability, disease prevalence and, perhaps most obviously, on food availability.

In the tropics, rainfall is the main determinant of primary productivity (Toledo *et al.*, 2011) and it also correlates positively with the nutritional quality of plant items (van Soest, 1982). Since the majority of the baboon diet, and that of most other primates, consists of plant matter, a link between rainfall and food availability, energy intake and condition may be assumed. Many of the findings of this thesis support this idea: for the Gashaka baboons higher levels of rainfall were associated with less time spent in energetically costly activities, i.e. feeding and travelling (chapter 3), lower energy expenditure rates without reduced energy intake rates (chapter 3), and higher urinary C-peptide levels (chapter 4), all of which suggest that food abundance and/or quality was positively correlated with rainfall. However, in contrast to this, heavy rainfall was associated with elevated glucocorticoids, suggesting that rainfall may act as a severe stressor to the baboons, and the relationship between energy intake rate and rainfall

varied according to season, with a significant negative relationship during the wet season. These findings are consistent with the idea that the link between rainfall and productivity is only present at Gashaka when rainfall is relatively low but still variable (i.e. at the transitions between seasons) and that the periods of very heavy rainfall during the wet season have little impact on productivity and therefore food availability (Schuur, 2003). The finding that glucocorticoids correlate positively with rainfall suggests that heavy rainfall has detrimental effects on the baboons' health, probably due to increased disease risk (Freeland, 1976), a proposal also supported by the fact that mortality during the year of this study (2009) and in previous years (2002-2006: Higham, 2006) has been strongly associated with the wet season. Together, these findings suggest that rainfall influences the Gashaka baboons' condition in two contrasting ways. Whilst initial increases in rainfall increase food availability and quality, allowing the baboons to sustain their energy intake rates at a reduced expenditure level, after a certain point, further increases in rainfall either do not increase productivity (e.g. Schuur, 2003) or the increase in productivity does not benefit the baboons, who already have sufficient food. Instead, the effect of further increases in rainfall appears to be negative, increasing stress levels and mortality, probably due to an increased disease burden. This cost of heavy rainfall, experienced by the Gashaka baboons, may well reflect the fact that the habitat in this region is at a geographical and ecological extreme for this species, whose physiology may not be well adapted to cope with the high disease burden associated with the unusually high rainfall (Semple *et al.*, 2002; Higham *et al.*, 2009a).

Little consistent influence of temperature on the baboons' condition could be identified and what could was most likely as a result of its correlation with vine-fruit availability. Vine-fruit availability, which represented the most temporally variable component of

total-fruit index, exhibited significant correlations with several of the condition variables. Both calculated energy intake and expenditure rate were positively associated with vine-fruit availability, which could suggest that the baboons are adopting an energy minimising strategy when food availability is low (by compensating for low energy intake, due to low food availability, with low energy expenditure) but adopting an energy maximising strategy when food availability is high (achieving high energy intake, by elevating energy expenditure rates, when food availability is high). During the dry season the predicted positive association between vine-fruit index and UCP level was also found, consistent with the idea that greater food availability enhances condition. In contrast, during the wet season there was a negative relationship between vine-fruit index and UCP level. This finding provides further support for the idea that food availability has a substantial influence on the baboons' condition when rainfall is relatively low, i.e. during the dry season, but that other factors, e.g. disease risk, are likely to have more influence on condition when rainfall is very high.

7.2. Relevance and implications of findings

7.2.1. Gashaka as a marginal habitat for baboons

One of the main reasons for studying the Gashaka baboons was that they represent a geographical and climatic outlier in comparison to the well studied baboon populations of southern and eastern Africa (Ross *et al*., 2011). By providing data on energy intake and expenditure rates, UCP, GC and progesterone levels from a population in, what is for baboons, a marginal habitat and one in which crop-raiding occurs, this study contributes to the understanding of the adaptive flexibility of this species both in terms of habitat variation and baboon-human interaction. As well as being of evolutionary interest, this is important for assessing the potential impact of environmental change as a result of climate change and the direct impact of human population increase, which

has relevance not only for baboons but also for other more vulnerable species which are facing similar pressures, but which may not be so easy to study (Warren *et al.*, 2011).

The findings of this study have, in particular, highlighted how the nature of the relationship between rainfall and individual condition depends on the degree of rainfall. Low to moderate rainfall increases primary productivity, food availability and, therefore, condition, whereas very high levels of rainfall increase stress levels and infant mortality, probably due to increased disease prevalence. Data from the current study have contributed towards the first demonstration of a positive association between rainfall and stress in a non-human primate (MacLarnon *et al.*, 2010). The findings of this study also suggest that the nature of the relationship between the climate an animal experiences and its condition, depends on how well adapted the animal is to that climate. In this study it was proposed that the negative effects of heavy rainfall on the Gashaka baboons' condition and survival were associated with the fact that the habitat at Gashaka represents a climatic and habitat extreme for baboons, for which they may not be so well adapted physiologically. This idea may have important implications for conservation. Habitat loss, degradation or fragmentation may force species to move into suboptimum habitats leading to exposure to novel climatic and ecological conditions. If there is not sufficient time for that species' immune system to adapt to the new conditions, the survival of the population may be threatened.

7.2. 2. The study of crop-raiding behaviour

The opportunity to compare a crop-raiding and an entirely wild-feeding troop of baboons was what first drew researchers to study the Gamgam and Kwano troops at Gashaka. The current study has built on previous research on these two troops, which demonstrated differences in activity budgets and reproductive output (Warren *et al.*,

2011), by providing further evidence that crop-raiding behaviour is associated with a relative improvement in condition in a population of baboons living at the edge of their environmental distribution. As anthropogenic habitats become more common across Africa and forests continue to be replaced by agriculture, baboons may be one of the very few species to benefit from human habitat disturbance, because of their adaptability and willingness to crop-raid (Warren *et al*., 2011). However, this kind of behaviour inevitably increases conflict between humans and non-human primates and consequently can make conservation of primates more difficult (Hill, 1997; Strum, 2010; Warren *et al*., 2011). Studies, such as this, which investigate the driving factors behind this behaviour and the costs and benefits for the crop-raiding animal, are therefore important if this conflict is to be better understood and ameliorated. Crop-raiding has been observed in a wide variety of primate species (Warren *et al.*, 2011) and, although baboons are not themselves threatened, the findings of this study could provide insights for more difficult to study, more at risk primate species, which also engage in crop-raiding (e.g. mandrills: Lahm, 1995; Zanzibar red colobus monkeys: Siex & Struhsaker, 1999; Pig-tailed macaques: Linkie *et al.*, 2007; Buton macaques: Priston *et al.,* 2012).

Although many studies have demonstrated a beneficial effect of food-enhancement on primate activity budgets and behaviour, in terms of reduced feeding/ travel time and increased resting time (see references in chapter 1 and section 3.3.1), the current study is the first to examine the effect of food-enhancement on the energy intake and expenditure of individual animals and the first to provide this kind of evidence for an energetic advantage of food-enhancement. This is also the first study, to my knowledge, to provide evidence for a reduction in glucocorticoid levels associated with food-enhancement in a free-ranging non-human primate, in contrast with the prediction that

increased conflict with humans should increase stress amongst raiding animals. This finding has particular relevance since increased stress is often cited as one of the costs associated with food-enhancement and in particular with crop-raiding in non-human primates (Fa, 1991; Chapman *et al.*, 2006; Higham, 2006; LaFleur and Gould, 2009) and other animals (Ahlering, *et al*, 2011).

7.2.3. Application of urinary C-peptides methods

The measurement of urinary C-peptides has previously been used to examine the link between diet, energy balance and condition in apes and in humans (Polonsky *et al.* 1988; Yoshida *et al.* 2006; Sherry and Ellison 2007; Deschner *et al.* 2008; Emery Thompson and Knott 2008) and more recently in monkeys (Girard-Buttoz *et al.*, 2011; Higham *et al.*, 2011a). The current study has built on this previous research by demonstrating that this method can be used effectively in baboons, which are important due to their wide geographical and ecological distributions and due to their use as a model organism (Jolly, 2001). This study is also the first to examine UCPs in relation to weather patterns, demonstrating a positive relationship with rainfall; the first to examine the effect of female rank on UCP levels, unexpectedly demonstrating the highest levels amongst low ranking females; and one of the first to use filter paper to store urine without freezing. This study has, therefore, helped to further the understanding and applicability of this novel technique.

7.2.4. Estimation of individual primates' energy intake and expenditure

Baboons are among the most extensively studied of the non-human primates, partly due to their geographic and ecological diversity, reflected by considerable variation in diet, life-history traits and social systems (e.g. Dunbar 1992). But despite a plethora of studies on baboon diet only a handful have investigated individual nutrient intake

(Barton and Whiten 1993; Altmann 1998). Investigation of individual energetic intake has been even more neglected with just three studies, all from the same, Amboseli study site (Stacey 1986; Muruthi *et al.* 1991; Altmann 1998). Studies of this kind involving other primate species are also rare (but see: Knott 1998; Wasserman and Chapman 2003; Vogel 2005; Conklin-Brittain *et al.* 2006; Emery Thompson and Knott 2008; Rothman *et al.* 2008). Studies, such as this, which provide detailed nutritional analysis of primate foods may contribute to the understanding of the relationship between primates and their ecological community and habitat as well as the link between primate feeding behaviour and sociality, factors which could have significant impact on the design and implication of primate conservation policy (Robbins and Hohman, 2006). The current study provides the first dataset of this kind for the olive baboon subspecies and for the poorly studied West African forest baboon.

7.2.5. Relationship between energetics, glucocorticoids and progesterone

The finding that Gamgam, the food-enhanced troop, exhibited lower progesterone levels than Kwano troop, alongside elevated energy intake rates and lowered glucocorticoid levels, may help to explain the link between food-enhancement and increased reproductive output of Gamgam troop. A possible scenario is that the energetic advantages of crop-raiding reduce the incidence and costs of disease for the Gamgam baboons relative to the Kwano baboons. The relatively lower food availability and higher disease risk of the Kwano baboons could then lead to relatively elevated glucocorticoid levels and an associated increase in progesterone levels, perhaps due to adrenal production (Plotka *et al.,* 1983). Elevated progesterone levels during inappropriate cycle phases (e.g. follicular development) may therefore underlie the relatively lower reproductive output of Kwano troop relative to Gamgam troop. This finding highlights the fact that the relationships between energetics, progesterone levels

and reproductive success are far from simple and it has clear implications for studies that assume a positive association between progesterone levels and conception probability (e.g. Ellison, 2003).

7.3. Limitations of current study and future research

One major limitation of the current study is that the results are correlational in nature. As such, the findings consist only of associations between the various measurements and it is not possible, from these results, to determine the causal direction of these relationships or whether a correlation is being driven by a third, unmeasured variable. The other major limitation of this study is that many of the findings relate to comparisons of just two troops, the crop-raiding Gamgam troop and the wild-feeding Kwano troop, with the implicit assumption that an examination of the difference between the two troops is equivalent to an examination of the effects of food-enhancement. Although Gamgam troop's crop-raiding behaviour does appear to represent the major difference between the two troops, since they belong to the same population and experience similar climate and habitat due to their geographical proximity, other differences do exist (e.g. group size, habitat structure) which means that the results of this study must be interpreted with caution. Future work could help elucidate these relationships further by including experimental elements such as manipulating energy intake (e.g. by supplementing the diets of certain animals) or hormone levels (e.g. treatment with exogenous glucocorticoid or progesterone sources).

Many of the findings of the current study provide support for the idea that disease risk may be an important influence on the Gashaka baboons' condition and reproductive output. A future study could examine how environmental factors such as rainfall and food availability influence disease prevalence or poor health as well as the impact of

disease on energetics, stress levels and reproductive output by employing a multifaceted health evaluation system, as used by Krief *et al.* (2005) with wild chimpanzees. This system involves non-invasive monitoring of the health of individual animals via several methods including clinical observations (e.g. visual examination for problems with respiratory, digestive, reproductive and locomotive functions by a vet or after training from a vet), parasitological analysis of faecal samples and urine analysis. Urine dipsticks could be used which allow various indicators of health to be measured non-invasively in the field, including indicators of infection and disease (e.g. the pH and the presence of leucocytes, blood, haemoglobin, protein or nitrate), hydration (specific gravity) and severe negative energy balance (ketones) (Krief *et al.,* 2005). Remote weighing of the animals using baited scales (Cooper *et al.*, 2004) alongside photogrammetry methods (Kurita *et al.,* 2011) could also be used in order to any track changes in body mass and BMI associated with weather patterns, food availability, energetics, hormone levels and health. These methods could also be used to examine the consequences of Gashaka baboons' apparent failure to adjust their energy intake and expenditure in response to the elevated costs of lactation.

The unexpected finding that progesterone levels correlated positively with glucocorticoid levels and that food-enhancement appeared to be associated with lowered levels of these two hormones warrants further investigation. An examination of the relationship between progesterone and glucocorticoid levels within individual females throughout different reproductive states, during both conceptive and non-conceptive cycles, could help elucidate the relationship between these two hormones and their effect on reproductive function. In addition, oestrogen levels could be analysed, via the same enzyme-immunoassay methods used in the current study to analyse progesterone and glucocorticoid levels. This would be useful since, like progesterone with which it

also interacts, oestrogen influences reproductive function and varies with both energetic status and stress (Wasser, 1995).

Finally, since the main focus of this project was on the energetics of individual baboons, the only aspect of nutrition that was investigated was energetic intake. As discussed in section 1.1, as well as energy, animals require protein, vitamins and minerals in order to grow, survive and reproduce and these requirements are known to affect both food choice and population distribution patterns and also to vary between animals during different stages in their life-history. An investigation of the nutritional intakes of individual baboons, which would be possible using the data collected for the determination of physiological energy content in this study (e.g. protein, fat and fibre content of food items), may uncover more differences between the diets of animals from the two troops, in different reproductive states, of different ranks and throughout the year.

7.4. Summary of conclusions

By utilising several different non-invasive methods, this project has demonstrated how the energetics and condition of female olive baboons vary both within and between troops and across time. The findings of this study have clear relevance to the fields of primate energetics, reproductive-endocrinology and conservation. Considerable evidence for a positive effect of food-enhancement, via crop-raiding, on the baboons' activity budgets, energy intake rates, and stress and reproductive hormone levels was presented as well as for the idea that food-enhancement buffers animals against environmental influences. Within troops, pregnant, but not lactating, animals adjusted their activity levels and energy intakes to compensate for the extra costs of reproduction, although, neither category of animal showed physiological signs of

energetic stress. Little impact of rank on the energetic or condition measures was detected, perhaps reflecting the lack of a strong dominance hierarchy amongst the Gashaka baboons. Rainfall was identified as a considerable but variable influence on the baboons' behaviour and condition, correlating with food availability and energy intake at low levels, and with stress levels and mortality at high levels. Future work could extend this research by performing experimental manipulations (e.g. of food intake or hormones levels), measuring health and disease parameters amongst the baboons, or by carrying out a more detailed investigation of the hormone levels of individual animals throughout conception, pregnancy and lactation.

APPENDICES

Appendix 1.

Latin names of animals referred to by common name in the text

Class Mammalia

Order Primates

Chimpanzee	*Pan troglodytes*
Bonobo	*Pan paniscus*
Western Gorilla	*Gorilla gorrilla*
Eastern Gorilla	*Gorilla beringei,*
Bornean orangutan	*Pongo pygmaeus*
Olive baboon	*Papio hamadryas anubis*
Yellow baboon	*Papio hamadryas cynocephalus*
Chacma baboon	*Papio hamadryas ursinus*
Guinea baboon	*Papio hamadryas papio*
Hamadryas baboon	*Papio hamadryas hamadryas*
Mandrill	*Mandrillus sphinx*
Vervet monkey	*Chlorocebus pygerythrus*
Sykes' monkey	*Cercopithecus mitus albogularis*
Rhesus macaque	*Macaca mulatta*
Long-tailed macaque	*Macaca fascicularis*
Barbary macaque	*Macacus sylvanus*
Bonnet macaque	*Macaca radiate*
Toque macaque	*Macaca sinica*
Pig-tailed macaque	*Macaca nemestrina*
Buton Macaque	Macaca ochreata brunnescens
Black and white colobus monkey	*Colobus guereza*
Red colobus monkey	*Procolobus rufomitratus*
Ugandan red colobus monkey	*Piliocolobus tephrosceles*
Zanzibar red colobus monkey	*Procolobus kirkii*
Phayre's leaf monkey	*Trachypithecus phayrei*
Hanuman langur	*Semnopithecus entellus*
Tufted capuchin	*Cebus apella*
White-faced capuchin	*Cebus capucinus*
White faced saki	*Pithecia pithecis,*

Black howler monkey	*Alouatta pigra*
Common marmoset	*Callithrix jacchus*
Black tufted-ear marmoset	*Callithrix kuhli*
Cotton-top tamarind	*Saguinus oedipus*
Ring-tailed lemur	*Lemur catta*
Fat-tailed dwarf lemur	*Cheirogaleus medius*

Superorder Ungulata

African elephant	*Loxodonta africana*
Red deer	*Cervus elaphus*
Mule deer	*Odocoileus hemionus*
White-tailed deer	*Odocoileus virginianus*
Pampas deer	*Ozotoceros bezoarticus*

Order Carnivora

Spotted hyena	*Crocuta crocuta*
Grizzly bear	*Ursus arctos*
Black bear	*Ursus americanus*
Elephant seal	*Mirounga angustirostris*

Order Rodentia

Degu	*Octodon degus*
Yellow-pine chipmunk	*Tamias amoenus*
Cascade golden-mantled ground squirrel	*Spermophilus saturatus*

Class Aves

Kittiwake	*Rissa tridactyla*
Wandering albatross	*Diomedea exulans*
Florida scrub-jay	*Aphelocoma coerulescens*
Barn swallow	*Hirundo rustica*
Song sparrow	*Melospiza melodia*

Appendix 2

DEFRA Product of Animal Origin import licences

First Licence (POAO/2009/044, valid until 27/07/2009)

European Communities Act 1972
The Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended)

Import Authorisation

defra
Department for Environment Food and Rural Affairs

Authorisation No.
POAO/2009/ 044

The Secretary of State for Environment, Food and Rural Affairs, in accordance with regulation 3(2) of the Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended) authorises:

name and full postal address	School of Human & Life Sciences Whitelands College Roehampton University Holybourne Avenue London Postcode SW15 4JD

to land in England in accordance with the conditions set out below

Product	Faeces and Urine derived from Wild Baboon
from (country of origin)	Nigeria
at (port of entry)	Heathrow Airport
until (date of expiry)	27/07/2009

Unless amended, suspended or revoked by the Secretary of State by notice to the person to whom it is issued.

Dated 28/01/2009 Signed

Officer of the Department for Environment, Food and Rural Affairs

Conditions attached to this Authorisation

1. This authorisation is valid for **multiple consignments** and the net weight per consignment must not exceed 15kg.
2. The products must remain in their original wrapping at all times until their arrival at: School of Human & Life Sciences, Whitelands College, Roehampton University, Holybourne Avenue, London, SW15 4JD.
3. The consignment shall be taken directly from the port of entry to the above address.
4. The Divisional Veterinary Manager at Riegate Animal Health Office (Tel: 01737 242242 Fax: 01737 241189) must be advised of the arrival of the consignment in England.
5. The consignment, or its packaging, must not be allowed to come into contact with any ruminating animals, swine, poultry or horses.
6. Immediately on arrival, all outer packaging shall be destroyed by incineration at Polkacrest Ltd, Platt Industrial Estate, Maidstone Road, Platt, Sevenoaks, Kent, TN15 8JN.
7. **None of the material to which this authorisation relates shall be used for human consumption under any circumstances.**
8. On completion of the testing any residues of the material and the remainder of the packaging shall be incinerated at the address stated in paragraph 6.
9. The importer must confirm in writing to the address below within 7 days of the incineration taking place that the above conditions have been adhered to.

POAO 2 (5/06)

Second Licence (POAO/2009/438, valid until 31/01/2010)

Authorisation No: POAO/2009/438
Revokes Authorisation POAO/2009/355

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS

European Communities Act 1972

THE PRODUCTS OF ANIMAL ORIGIN (THIRD COUNTRY IMPORTS) (ENGLAND) REGULATIONS 2006 (AS AMENDED)

The Secretary of State for Environment, Food and Rural Affairs, by this authorisation issued under the terms of Regulation 4 of the Products of Animal Origin (Third Country Imports) (England) Regulations 2006 (as amended) authorises:

School of Human & Life Sciences Whitelands College Roehampton University Holybourne avenue London SW15 4JD	Name and full postal address

subject to and in accordance with the conditions set out below, the landing in England of:

Faeces and urine derived from wild baboons, intended for particular studies or analyses	Product

from

Nigeria	Countries of origin

at

Heathrow Airport	Ports of entry

until

31 January 2010	Expiry Date

Dated: 25 September 2009

Official of the Department for Environment, Food and Rural Affairs

Authorisation No: POAO/2009/438
Revokes Authorisation POAO/2009/355

Conditions attached to this authorisation

1. This licence/authorisation is valid for multiple consignments and the weight per consignment must not exceed 15kg.

2. Each consignment must be accompanied by:

- commercial/shipping documents providing the name and address of consignor and consignee, type of product and quantities;

- a declaration that the products are **not** derived from animals known or suspected to be infected with a pathogen which causes a notifiable disease to which the animals from which the products are derived are susceptible according to European Regulations* or the Animal Health Regulations of the exporting country, nor do the products originate from animals in a premises or region or zone of a country that is subject to official restrictions due to a notifiable disease to which the animals are susceptible according to European or other National Animal Health Regulations;

  *Council Directive 82/894/EEC of 21 December 1982 (as amended) on the notification of animal diseases within the Community.

- A copy of this import licence/authorisation.

- A declaration that the faecal samples are stored in >90% ethanol.

3. The packaging must be clearly labelled to indicate the nature of the product and that this is intended for *in vitro* use at the University of Roehampton for research;

4. The samples must be worked on and stored in facilities working to containment level 2 until study is complete at which time samples shall be destroyed.

5. The samples and material derived from the samples shall be used for *in vitro* use only.

6. The consignment including its packaging, must not be allowed to come into contact with any ruminating animals, swine, poultry or horses.

7. Immediately on arrival, all outer packaging shall be destroyed by incineration at a premises that has been approved for the disposal of animal by-products (see note 3 below).

8. Samples or sub-samples deriving from these must not be supplied to other premises in the UK.

9. . On completion of the testing any residues of the material and the remainder of the packaging shall be incinerated at a premises that has been approved for the disposal of animal by-products (see note 6 below). Records of the disposal of the material and packaging must be kept for two years. The importer must confirm in writing to the address below within 7 days of the incineration taking place that the above conditions have been **adhered to.**

CONTACT FOR FURTHER INFORMATION

Animal Health Divisional Office
Animal Health Import Team
Beeches Road
Chelmsford
Essex
CM1 2RU

Tel: 01245 358383
Fax: 01245 351162
e-mail AHITChelmsford@animalhealth.gsi.gov.uk

Appendix 3

Hormone analysis protocols not detailed in the text

Double extraction of hormone metabolites from faecal samples

To initiate the double extraction methods the faecal samples were transferred into pre-weighed and labelled 30ml centrifuge tubes; all undigested matter was discarded. Samples were transferred and broken up using a glass rod and the original collection bottle and glass rod were rinsed with 80% methanol into the centrifuge tube to ensure that all faecal matter had been transferred. Samples were then agitated on a tube shaker (multi-tube vortexer VWR, VX-2500) for 10 minutes after which the sides of the tubes were rinsed with methanol to remove any solid matter into the solution. The tubes were then centrifuged at 4500 rpm for 12 minutes (Centrifuge: Heraeus Labofuge 400R). The resulting supernatant was poured off into a second set of pre-weighed, labelled tubes. 5 ml of 80% methanol was added to the remaining solid matter in the original centrifuge tubes, which was re-suspended using the glass rod, and the sequence of agitating and centrifuging the samples was repeated. The additional supernatant was then added to that already present in the 2nd set of centrifuge tubes, the total volume was recorded and the tubes were stored at 5^{o}C until the assay procedure began. The original set of centrifuge tubes, containing the solid faecal matter, were placed in a 40^{o}C oven with their lids off and weighed periodically until their mass stabilised. At this point the original tube mass was subtracted from the current total mass to give the dry mass for each faecal sample.

Enzyme Immunassay protocol for analysis of hormone metabolites

The assay procedure took place over two days. On the first day the standard curve and sample dilutions were prepared using assay buffer. The pre-coated plate was washed

four times with wash buffer (containing Phosphate Buffered Saline and Tween 20) using a plate-washer (Wellwash 4MK2 Thermo Labsystems), the plate was then struck onto absorbent paper to ensure all wells were free from water. The appropriate quantities of assay buffer, standard curve dilution, quality control or diluted sample were then dispensed into the appropriate well using a precision pipette (Biohit M100 or M200) fitted with disposable pipette tips. Figure A.2. demonstrates the layout of the assay plate. Stock Biotin labelled steroid, diluted with 5.6ml of assay buffer, was then added to each well using a multichannel pipette (Biohit M300). After this, stock steroid specific antibody, diluted with 5.6ml of assay buffer, was added to all except the blank wells using the multipipette. The plate was then covered with clingfilm and gently agitated for approximately 5 minutes on a plate shaker (Thermo Scientific Denley MiniMix) and then incubated at 5^{o}C overnight.

Figure A3.i. Layout of PdG an 5β-Adiol assay plates

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	BL	1	1	6	6	14	14	20	20	26	26
B	Z	Z	2	2	7	7	15	15	21	21	27	27
C	S1	S1	S7	S7	8	8	H	H	22	22	28	28
D	S2	S2	S8	S8	9	9	L	L	23	23	29	29
E	S3	S3	S9	S9	10	10	16	16	24	24	30	30
F	S4	S4	3	3	11	11	17	17	H	H	31	31
G	S5	S5	4	4	12	12	18	18	L	L	32	32
H	S6	S6	5	5	13	13	19	19	25	25	33	33

BL=Blank, Z=Zero, S1-9= Standards [for PdG: 6.25, 12.5, 25, 50, 100, 200, 400, 800 and 1600 pg/50µl; for 5β-Adiol: 0.6, 1.21, 2.43, 4.87, 9.75, 19.5, 39, 78 and 156 pg/50µl], 1-32= Samples, H= QCH, L=QCL

The following day the plate was washed as before and 150µl of streptavidin–peroxidase (25µl of 1mg/1ml stock diluted with 18ml of assay buffer) was added to each well using

the multipipette. The plate was then covered with cling film and incubated at room temperature on the plate shaker for 30 minutes. The plate was then washed for a final time and 150µl of TMB solution (195µl TMB plus 20ml Working Substrate Buffer) was added to each of the wells using the multipipette. The plate was then covered and placed back on the plate shaker where it was left to incubate for between 45 and 90 minutes until an appropriate colour change in the Zero wells (transparent to deep blue) was observed. At this point 50µl of 2M sulphuric acid was added to each well using the Eppendorf Research Pro 1200 to stop the reaction. An automated plate reader (Multiskan Ascent, Thermo Labsystems) was then used to measure the optical density of each well. The metabolite concentration in each well was then determined from the standard curve by the Ascent software.

Appendix 4

Procedures and calculations for determining acid detergent fibre (ADF) and acid detergent lignin (ADL) content of food item samples

ADF procedure

Following the NDF procedure (section 2.2.3), the sample bags were returned to the digestion vessel which was then filled with two litres of Acid Detergent Solution, consisting of 1.00*N* Sulphuric acid and Cetyl trimethylammonium. The samples were then heated and agitated in the solution for 60 minutes after which the samples were rinsed three times with boiling distilled water. The samples were then soaked in acetone and dried for two hours. The Sample bags were then brought to room temperature in the desiccator and weighed.

ADL procedure

After the ADF procedure the sample bags were placed in a beaker along with 24.00*N* Sulphuric acid and kept submerged with a smaller beaker, which was also used to agitate the bags at 30 minute intervals. After three hours the acid was poured off and the bags were rinsed with boiling distilled water until the pH became neutral. The bags were then soaked in acetone, as before, and dried in the oven for four hours. After cooling to room temperature in the desiccator the bags were weighed once more and then placed in pre-weighed ceramic beakers which were heated to 525^{o}C for 3 hours. The mass of the remaining ash was calculated after the ceramic beakers had returned to room temperature in the desiccator.

Calculating fibre content

The percentages of NDF, ADF, ADL, cellulose and hemicellulose for each sample were calculated using the following equations:

(a) $C = \frac{Mb_{ag}\,(M_{burn} - M_{cup})}{Mb_{ag}}$

(b) $NDF\% = \frac{(M_{NDF} - (Mb_{ag} \times C) - (M_{burn} - M_{cup})}{M_s} \times 100$

(c) $ADF\% = \frac{(M_{ADF} - (Mb_{ag} \times C) - (M_{burn} - M_{cup})}{M_s} \times 100$

(d) $ADL\% = \frac{(M_{ADL} - (Mb_{ag} \times C) - (M_{burn} - M_{cup})}{M_s} \times 100$

(e) Cellulose % = ADF% - ADL%

(f) Hemicellulose % = NDF % - ADF %

M_{NDF} = Mass of sample and bag after NDF analysis, M_{ADF} = Mass of sample and bag after ADF analysis, M_{ADL}= Mass of sample and bag after ADL analysis, Mb_{ag} = Mass of sample bag, C = bag correction factor, M_{burn} = Mass of sample and cup after burning, M_{cup} =Mass of sample cup, M_s = Mass of sample

Appendix 5

Components for the calculation of item specific energetic intake rates

Table A5.i. Energetic content and feeding rates for food items eaten by the Gashaka baboons between March and December 2009 (continued over page)

			% feeding time spent on item		Details from energetic content analyses (source of sample, nutrient values (%) and calculated energy content of sample):							Feeding rate details:				
Item no.	**Species[a]**	**Part**	**Kwano**	**Gamgam**	**Source**	**NDF[b]**	**Fat**	**protein**	**Ash**	**TNC[c]**	**Energy content [d]**	**Source**	**Items/ min**	**Item mass**	**Mass/ min**	**Energy intake rate[e]**
1	*Afromomum* spp.	Stem	0.00	0.99	SG	58.64[f]	0.73[f]	8.93	16.99	14.71	537.11	MSB			2.28	12.22
2	*Afromomum* spp.	USO	0.00	0.08	SG	54.43	3.42	7.27	15.75	19.13	669.91	MU			0.30	1.98
3	*Annona senegalensis*	Flower	0.04	0.20	SK	64.72	2.54	17.69	5.91	9.15	648.76	MF			0.66	4.28
4	Barley grass	Seed	0.00	0.55	SG	72.49	2.37	10.67	7.29	7.18	529.67	MGS			2.55	13.52
5	Barley grass	Seed	0.32	0.00	SK	73.85	2.11	9.70	7.57	6.76	503.35	MGS			2.55	12.85
6	Blue vine flower	Flower	0.00	0.31	SG	34.67	4.47[f]	25.57	9.03	26.26	1035.59	MF			0.66	6.82
7	*Bridelia ferruginea*	Fruit	0.00	1.51	SG	49.00	6.46	7.75	3.66	33.13	999.58	O	25.82	0.09	2.40	24.02
8	*Bridelia ferruginea*	Fruit	0.27	0.00	SK	52.36	5.29	8.11	4.05	30.18	923.79	O	25.82	0.08	2.14	19.74
9	*Cussonia arborea*	Fruit	0.10	0.00	SK	50.31	8.78	8.73	9.68	22.49	923.53	MFS			2.65	24.47
10	*Daniellia oliveri*	Flower base	0.00	0.28	SG	20.44	5.85	21.17	7.07	45.47	1301.75	O	20.00	0.05	0.94	12.30
11	*Daniellia oliveri*	Flower base	0.49	0.00	SK	21.67	5.03	22.14	6.89	44.27	1270.08	O	20.00	0.02	0.37	4.74
12	*Elaeis guineensis*	Nut (outer covering)	0.00	0.00	SK	18.86	64.64	3.67	2.78	10.05	2526.92					
13	*Elaeis guineensis*	Nut (inner flesh)	0.00	0.00	SK	24.59	53.76	11.41	2.08	8.16	2238.95					
12/13[g]	*Elaeis guineensis*	Entire nut	17.46	1.86	SK						2402.67	O	2.76	1.06	2.92	70.05
14	*Erythrophleum suaveolens*	Seed (fresh)	22.75	3.26	SK	17.33	10.72	39.40	4.93	27.62	1431.30	O	2.57[h]	0.30	0.77	11.06
15	*Erythrophleum suaveolens*	Seed (dry)	0.01	3.56	SG	37.77	4.50	13.49	3.56	40.69	1109.18	O	20.33	0.55	11.09	122.98

Table A5.i. Continued from previous page.

Item no.	Species[a]	Part	Kwano	Gamgam	Source	NDF[b]	Fat	protein	Ash	TNC[c]	Energy content [d]	Source	Items/ min	Item mass	Mass/ min	Energy intake rate[e]
16	*Ficus polita*	Fruit	0.39	0.00	SK	39.05	2.42	5.13	6.84	46.56	1016.71	MFM			10.29	104.58
17	*Ficus natalensis*	Fruit	0.79	0.51	SK	44.01	8.28	5.77	6.96	34.97	1053.34	O	23.50	0.08	1.82	19.18
18	*Ficus sur*	Fruit	0.15	0.47	SK	40.84	6.51	9.47	10.56	32.62	997.54	O	5.43	1.03	5.57	55.57
19	Unknown grass	Stem base	5.59	5.48	SK/G	63.27	0.71	10.38	15.55	10.09	492.58	O	16.54	0.08	1.24	6.12
21	Unknown grass	Leaf blade	0.80	0.92	SK	62.86	1.31	17.49	9.86	8.48	587.37	O	30.67	0.04	1.11	6.51
23	*Ficus* sp. "Large green fruit"	Fruit	0.00	0.00	SK	38.08	1.85	4.86	8.18	47.04	998.34					
24	*Nauclea latifolia*	Fruit	0.00	1.00	SG	42.95	7.79	6.02	7.31	35.92	1052.53	O	0.88	14.41	12.74	134.13
26	*Nauclea latifolia*	Fruit	1.45	0.00	SK	43.89	8.75	8.01	5.19	34.15	1087.84	O	0.88	10.83	9.58	104.22
27	*Pandanus candelabrum*	Fruit	0.24	0.00	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFL			36.56	368.47
28	*Panicum maximum*	Stem	0.00	0.52	SG	54.01	0.74	16.47	25.21	3.56	450.66	MSB			2.28	10.26
29	*Parinari excelsa*	Fruit	0.31	0.13	SG	23.03	0.31	4.56	3.50	68.60	1257.02	MFM			10.29	129.29
30	*Parkia biglobosa*	Seed	0.69	0.30	SK/G	10.81	15.83	50.19	4.70	18.47	1597.68	O	10.02	0.15	1.49	23.76
31	*Parkia biglobosa*	Pith	0.43	3.01	SK	17.68	0.53	5.54	4.35	71.89	1319.83	O	9.00[h]	3.36	30.20	398.62
32	*Piliostigma thonningii*	Seed	0.00	3.52	SG	41.70	1.41	18.95	3.72	34.23	982.27	O	0.51	1.51	0.78	7.63
33	*Piliostigma thonningii*	Seed	1.19	0.00	SK	37.42	1.41	17.53	3.33	40.31	1051.46	O	0.51[h]	0.67	0.35	3.65
34	*Prosopis africana*	Seed	3.54	1.44	SG	31.97	7.67	13.92	5.00	41.44	1224.47	O	1.20[h]	1.34	1.61	19.75
36	Red Berry 1	Fruit	0.00	0.38	SG	52.61	1.43	8.80	7.01	30.15	798.14	O	26.95	0.08	2.28	18.21
37	Red berry 1	Fruit	0.48	0.00	SK	56.70	2.11	5.88	7.65	27.66	749.53	O	26.95	0.13	3.48	26.06
38	Red berry 2	Fruit	0.26	0.00	SG	18.90	21.05	12.42	4.40	43.23	1669.22	O	36.81	0.06	2.05	34.17
39	*Rottboellia exaltata*	Seed head	0.00	11.25	SG	68.74	2.74	10.25	10.04	8.22	544.71	O	27.79	0.12	3.41	18.57
40	*Rottboellia exaltata*	Seed head	0.74	0.00	SK	71.76	1.66	8.09	11.40	7.10	464.94	O	27.79	0.06	1.69	7.88
41	Savannah Forb	Stem base	0.40	1.53	SG	58.64[e]	0.73[f]	8.91	39.71	7.99	164.57	O	25.00	0.13	3.31	5.45

Table A5.i. Continued from previous page.

Item no.	Species[a]	Part	Kwano	Gamgam	Source	NDF[b]	Fat	protein	Ash	TNC[c]	Energy content [d]	Source	Items/ min	Item mass	Mass/ min	Energy intake rate[e]
42	Savannah Forb	Leaf	0.11	0.03	SK	53.21	2.82	17.46	20.65	5.86	573.37	O	4.05	0.19	0.78	4.45
43& 44	Small Pod	Seed	0.94	5.03	SG	26.45	8.13	35.37	5.08	24.96	1262.09	TP			0.41	5.15
45	Tiboko Large	Fruit	0.60	3.66	SK	51.24	2.51	7.32	4.24	34.69	886.27	O	0.58	52.88	30.55	270.76
46	Tiboko Medium	Fruit	0.79	3.53	SK	57.80	2.16	7.80	3.94	28.30	791.91	O	0.57	23.18	13.25	104.91
47	Tiboko sp.	USO/ Root	2.09	0.00	SK	28.47	5.73	5.33	11.61	48.86	1147.52	O	1.43	0.21	0.30	3.40
48	*Tricalysia oligonvera/ oligoneura*	Fruit	0.12	0.44	SG	40.40	11.45	8.25	4.84	35.06	1192.14	O	24.00	0.15	3.61	43.08
49	Tuchi	Seed	3.56	0.03	SK	41.77	1.93	30.79	4.29	21.23	955.80	O	12.50[h]	0.03	0.41	3.90
50	*Uapaca togoensis*	Fruit	1.92	2.29	SK/G	41.94	0.92	2.84	4.72	49.59	988.35	O	14.27	0.50	7.09	70.04
51	*Vitex doniana*	Fruit	1.16	0.16	SG	15.06	0.58	2.50	14.14	67.71	1203.24	MFM			10.29	123.76
52	White Berry	Fruit	0.00	0.04	SG	60.62	2.37	8.32	4.26	24.43	750.13	O	26.31	0.04	0.93	6.95
53	White berry	Fruit	0.26	0.00	SK	55.83	2.74	8.23	4.61	28.59	818.27	O	26.31	0.03	0.70	5.73
54	*Xylopia* sp.	Seed	0.57	0.08	SK	56.56	23.01	11.97	1.88	6.58	1222.24	MS			4.45	54.39
55	*Zea mays*	Seed	0.00	0.96	SG	11.81	4.02	11.40	1.39	71.37	1502.48	O	0.24	120.12	28.43	427.14
57	*Mangifera indica*	Fruit	0.73	2.01	UK	8.03	0.47	2.56	2.24	86.70	1494.44	O	8.268[i]	3.34	27.58	412.23
58	*Musa sapientum*	Fruit	0.00	0.07	UK	4.00	0.17	4.90	4.00	86.93	1511.19	O	2.65	16.08	42.57	643.30
59	*Anogeissus leiocarpa*	Exudate	0.01	0.00	[1]						1206.88	[1]			1.71	20.64
60	*Anogeissus leiocarpa*	Fruit	0.02	0.00	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFM			10.29	103.67
61	*Anogeissus leiocarpa*	Leaf	0.00	0.96	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
62	*Annona senegalensis*	Fruit	0.11	0.75	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	O	4.45	7.84	34.88	351.55
63	*Apodytes dimidiata*	Fruit	0.05	0.00	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFS			2.65	26.70
64	Bush Yam	USO	0.44	0.00	[3]						1617.65103	LQ			0.99	15.94
65	*Cussonia arborea*	Exudate	0.01	0.00	[1]						1206.88	[1]			1.71	20.64

Table A5.i. Continued from previous page.

Item no.	Species[a]	Part	Kwano	Gamgam	Source	NDF[b]	Fat	protein	Ash	TNC[c]	Energy content [d]	Source	Items/ min	Item mass	Mass/ min	Energy intake rate[e]
66	*Ficus polita*	USO	0.10	0.00	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
67	*Ficus ovata*	Fruit	0.03	0.01	MFC	40.49	4.77	6.31	8.14	40.30	1016.48	O	7.50	7.84	58.77	597.38
68	*Ficus ovata*	USO	0.07	0.00	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
69	*Ficus* spp.	Fruit	2.02	3.88	MFC	40.49	4.77	6.31	8.14	40.30	1016.48	O	9.15	7.84	71.74	729.17
70	Goyabe	Fruit	0.16	2.81	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	O	3.63	7.84	28.47	286.95
71	Unknown grass	Seed	4.72	0.41	MGM	71.71	2.22	9.68	9.08	7.32	510.67	MGS			2.55	13.03
72	Hard Green Fruit	Seed	0.49	0.00	MS	43.93	6.55	21.83	5.56	22.13	1010.09	MS			4.45	53.59
73	Hard Green Fruit	Fruit	0.30	0.00	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFM			10.29	103.67
74	*Landolphia owariensis*	Fruit	0.06	0.07	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFS			2.65	26.70
76	*Landolphia* spp.	Fruit	0.18	0.14	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFS			2.65	26.70
77	*Pandanus candelabrum*	USO	0.08	0.00	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
78	*Pseudospondias microcarpa*	Fruit	0.75	0.17	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFM			10.29	103.67
79	*Syzygium guineense*	Fruit	0.08	0.07	MFC	40.49	4.77	6.31	8.14	40.30	1016.48	O	5.25	7.84	41.14	418.17
82	Tibiko Medium	Stem	0.01	0.00	MSB	58.64	0.73	11.17	24.37	5.09	411.23	MSB			2.28	9.36
83	Tiboko Small	Fruit	0.44	0.31	MT	54.52	2.33	7.56	4.09	31.50	839.09	MFM			10.29	86.31
84	Tiboko sp.	Seed	2.41	0.00	MT	54.52	2.33	7.56	4.09	31.50	839.09	O	16.67	0.86	14.31	120.06
85	Tiboko sp.	Fruit	0.99	1.51	MT	54.52	2.33	7.56	4.09	31.50	839.09	O			21.90	183.75
86	Tiboko sp.	Stem	0.02	0.00	MSB	58.64	0.73	11.17	24.37	5.09	411.23	MSB			2.28	9.36
87	*Vitellaria paradoxa*	Fruit	0.01	0.00	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFL			36.56	368.47
88	*Vitellaria paradoxa*	USO	0.07	0.00	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
89	*Vitex doniana*	Leaf	0.04	0.00	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
90	*Afzelia africana*	Seed	0.00	0.03	MS	43.93	6.55	21.83	5.56	22.13	1010.09	MS			4.45	53.59
91	*Borassus aethiopum*	Fruit	0.00	0.05	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFL			36.56	368.47

Table A5.i. Continued from previous page.

Item no.	Species[a]	Part	Kwano	Gamgam	Source	NDF[b]	Fat	protein	Ash	TNC[c]	Energy content [d]	Source	Items/ min	Item mass	Mass/ min	Energy intake rate[e]
92	*Colocasia esculenta*	USO	0.00	0.15	[3]						1587.56	LQ			0.99	15.64
93	*Daniellia oliveri*	Leaf	0.00	0.02	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
94	*Daniellia oliveri*	Seed	0.00	0.23	MS	43.93	6.55	21.83	5.56	22.13	1010.09	O	3.43	0.86	2.94	35.44
95	*Erythrophleum suaveolens*	Exudate	0.00	0.00	[1]						1206.88	[1]			1.71	20.64
96	Fafe (grass spp.)	Stem	0.00	1.09	19	63.27	0.71	10.38	15.55	10.09	492.58	MSB	20.77		2.28	11.21
97	*Ipomoea batatas*	USO	0.00	0.10	[3]						1623.73	LQ			0.99	16.00
98	*Manihot esculenta*	USO	0.00	0.35	[3]						1641.67	LQ			0.99	16.18
99	*Mucuna poggei*	Flower	0.00	0.15	MF	35.38	4.47	21.64	7.23	31.29	1064.04	MF	20.00		0.66	7.01
100	*Mucuna poggei*	Leaf	0.00	0.87	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
101	*Mucuna poggei*	Seed	0.00	0.33	MS	43.93	6.55	21.83	5.56	22.13	1010.09	MS			4.45	53.59
102	*Mucuna poggei*	USO	0.00	0.07	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
104	*Parinari excelsa*	Flower	0.00	0.02	MF	35.38	4.47	21.64	7.23	31.29	1064.04	MF	20.00		0.66	7.01
105	*Piliostigma thonningii*	Leaf	0.00	0.02	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
106	*Terminalia* spp.	Bark	0.00	0.05	[2]						399.26	LQ			0.99	3.93
107	*Terminalia* spp.	Leaf	0.00	0.44	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
108	*Terminalia* spp.	Seed	0.00	0.21	MS	43.93	6.55	21.83	5.56	22.13	1010.09	MS			4.45	53.59
109	*Tricalysia oligonvera/ oligoneura*	USO	0.00	0.01	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
110	Other/Unknown	Flower	0.07	1.05	MF	35.38	4.47	21.64	7.23	31.29	1064.04	MF	20.00		0.66	7.01
111	Other/Unknown	Fruit	1.17	6.21	MFA	43.73	5.19	7.04	6.29	37.75	1007.87	MFA			8.80	88.66
112	Other/Unknown	Leaf	1.54	1.24	ML	58.03	2.07	17.48	15.25	7.17	580.37	ML			0.94	5.47
113	Other/Unknown	Seed	0.55	0.83	MS	43.93	6.55	21.83	5.56	22.13	1010.09	MS			4.45	53.59
114	Other/Unknown	Stem	0.42	0.43	MSB	58.64	0.73	11.17	24.37	5.09	411.23	MSB			2.28	9.36

Table A5.i. Continued from previous page.

Item no.	Species[a]	Part	Kwano	Gamgam	Source	NDF[b]	Fat	protein	Ash	TNC[c]	Energy content [d]	Source	Items/ min	Item mass	Mass/ min	Energy intake rate[e]
115	Other/Unknown	Bark	0.17	0.25	[2]						399.26	LQ			0.99	3.93
116	Other/Unknown	Exudate	0.37	0.09	[1]						1206.88	[1]			1.71	20.64
117	Other/Unknown	USO	0.67	0.77	MU	41.45	4.58	6.30	13.68	33.99	908.71	MU			0.30	1.78
118	Other/Unknown	Part unknown	3.50	1.85	MA	42.48	7.06	12.99	7.98	28.22	1005.38	MA	13.22		7.15	71.49
119	Fungus	N/A	1.04	1.40	[4]						533.22	MA	13.28		7.15	38.12
120	Ant	N/A	0.69	4.13	[5]						723.72	O	35.84	[6]	0.07	0.52
121	Caterpillar	N/A	0.07	0.36	[7]						2281.39	O	19.50	[8]	2.14	48.76
122	Invertebrate	From Ground/ vegetation	2.15	4.17	[7]						1591.79	O	14.05	EM	4.09	65.06
123	Invertebrate	From water	2.20	0.54	[7]						1591.79	O	0.87	EM	0.25	4.04

Code	Description
a	'Species' represents Latin name (italicised), local name or name given by Emily Lodge for plants or the broad category the item belongs to for non-plant items e.g. 'ant' or 'fungus'. Latin and local names follow Warren 2003.
b	NDF = Neutral Detergent Fibre
c	TNC (Total non-structural Carbohydrate) = 100-NDF+CP+lipid+ash
d	Energy content (kJ/100g) = (Protein×14.23)+(TNC×16.40)+(Fat×35.02)+(NDF×2.44). Includes dry mass correction
e	Item specific rate of energy intake rate (kJ/min)= Energy content (kJ/g) * Mass feeding rate (g/ min)
f	Nutritional analysis not performed on this sample so mean value for the appropriate part and nutrient is used
g	The baboons eat both the outer layer and inner seed from palm nuts (*Elaeis guineensis* seeds) and the macronutrient content of these parts was analysed separately. The combined energetic content value is a weighted average of the two parts which accounts for the fact that the outer layer represents 56.85% of the total dry weight while the inner nut represents 43.15%
h	Item feeding rate is based on number of seed pods eaten rather than number of seeds
i	Item feeding rate is based on number of 'bites'
EM	'Estimated mass' of invertebrates, used mean of values from Wu Leung 1968 and McGrew 1974

LQ	Lower quartile of all observed mass feeding rates (value selected due to the personal observation that these items were eaten at a fairly slow rate)
SG	Sample collected from Gamgam
SK	Sample collected from Kwano
SK/G	Item collected from Kwano and Gamgam sites and pooled into one sample
MA	Mean of all values (uses energetic content data for all sampled items or mass feeding rate values for all items for which the rate was directly observed)
MF	Mean flower value
MFA	Mean fruit value (all sizes)
MFC	Mean *Ficus* spp. value
MFL	Mean large fruit value
MFM	Mean medium fruit value
MFS	Mean small fruit value
MGM	Mean grass species value
MGS	Mean grass seed head value
ML	Mean leaf value
MS	Mean seed value
MSB	Mean stem base value
MT	Mean 'tiboko' value
MU	Mean USO value
O	Observed feeding rate
TP	'Tuchi' pod value
UK	Energetic content of *Mangifera indica* (mango) and *Musa sapientum* (banana) fruit bought in the UK used
[1]	Values for 'Fever tree gum' from Altmann, 1998
[2]	Mean values for bark eaten by Gorillas in Calvert, 1985
[3]	Values for specific crop foods grown in Africa from Wu Leung, 1968. Specific values used for raw *Manihot esculenta* (cassava), *Ipomoea batatas* (sweet potato) and *Colocasia esculenta* (Cocoyam). Mean of these values used for 'bush yam'
[4]	Mean value of fungus eaten by primates from Hanson *et al.*, 2006
[5]	Mean energetic content of 8 adult ant species eaten by apes in Cameroon from Deblauwe and Janssens, 2008
[6]	Mass of *Dorylus (Anomma) nigricans* worker ant used from McGrew 1974, referenced In Schoning *et al.*, 2007

[7] Values from Lawel and Songonuga, 2006. Energetic content of 'Caterpillars' is mean of 5 caterpillar values given in paper. Energetic content of 'Invertebrates' is mean of three adult cricket and grasshopper values given in paper.

[8] Mass of 4cm caterpillar estimated from equation in Ganihar, 1997

Table A5.ii. Mean values of energy content (kJ/100g) used in estimation of values for non-sample food items.

Category			**Mean nutritional content** (g/100g)					
Code	**Description**	**Food items used to calculate means**	**NDF**	**Fat**	**Protein**	**Ash**	**TNC**	**Mean energy content (kJ/100g)**
MT	Tiboko fruit	45,46	54.52	2.33	7.56	4.09	31.5	839.09
MGS	Grass seed heads	4,5,39,40	71.71	2.22	9.68	9.08	7.32	510.67
MFC	*Ficus* spp. Fruit	16,17,18,23	40.49	4.77	6.31	8.14	40.3	1016.48
MFA	All Fruit	7,8,9,16,17,18,23,29,36,37,38,45,46, 48,50,51,52,53,24,26	43.73	5.19	7.04	6.29	37.75	1007.87
MU	USO	2,47	41.45	4.58	6.3	13.68	33.99	908.71
MS	Seed	4,5,32,33,34,39,40,43,44,49,54,15,14 ,30	43.93	6.55	21.83	5.56	22.13	1010.09
ML	Leaf	21,42	58.03	2.07	17.48	15.25	7.17	580.37
MSB	Stem base	1,19,28,41	58.64	0.73	11.17	24.37	5.09	411.23
MF	Flower/Flower base	3,6,10,11	35.38	4.47	21.64	7.23	31.29	1064.04
All	Mean value for all sampled items	All samples except crops (55,56,57,58)	42.48	7.06	12.99	7.98	28.22	1005.38

Table A5.iii. Mean values of mass feeding rate (g/min) used in estimation of values for items for which this was not directly observed.

Category			
Code	**Description**	**Food items used to calculate mean**	**Mean mass feeding rate (g/min)**
MF	Flower/Flower base	10,11	0.66
MFL	Large fruit (>2cm)	45,58	36.56
MFM	Medium fruit (2-5cm)	18,24,26,46	10.29
MFS	Small fruit (<5cm)	7,8,17,36,37,38,48,50,52,53	2.65
MFA	All fruit	45,58,18,24,26,46,7,8,17,36,37,38,48,50,52,53	8.80
ML	Leaf	21,42	0.94
MU	USO/ root	47	0.30
MS	Seed	15,30,32 (EG or grass seeds not included)	4.45
MSB	Stem base	19,41	2.28
MGS	Grass seed head	39, 40	2.55
MA	Mean value of all observed mass feeding rates	57,55,56,10,11,45,58,18,24,26,46,7,8,17,36,37,38,48,50,52, 53,42,21,14,31,33,34,49,47,12,13,15,30,32,39,40,19,41	8.61
LQ	Lower quartile of all observed mass feeding rates	Same as for MA	0.99

Appendix 6

Full results of GLMMs including non-significant results

Explanations of terms in tables:

- D = likelihood test statistic. The model to which the model of interest is compared for the likelihood ratio test is given in the 'comparison model' column.
- P values in **bold** are significant at the $p<0.05$ level, values in ***bold and italicised*** show a trend: $p<0.1$.
- AIC= Akaike Information Criterion, a measure of the models relative goodness of fit. Calculated using the following equation:
  AIC= 2*number of parameters in model – (-2*loglikelihood of the model)
- VPC= Variance Partition Coefficient. The amount of variation in the dependant variable's dataset explained by the model can be partitioned into that explained by the first fixed factor (e.g. focal or sample number) which represents within individual variation, and that explained by the second fixed factor (ID) which represents between individual variation, using the VPC, calculated using the following equation: VPC= Between individual variance / (Within individual variance + Between individual variance).
- Coefficient= for a continuous variable, this is the slope of the relationship between the explanatory and dependant variable and, for a categorical variable, this is the difference between the average values for two categories within a categorical variable (e.g. average % time spent resting for Kwano troop minus that for Gamgam troop)
- s.e.= Standard error of coefficient
- * indicates that the interaction effect between the two mentioned explanatory variables is included in the model.
- In general, z tests were only performed when the model of interest is significantly better fitting than the simpler model to which it was compared (i.e. if the D statistic is significant or marginally significant).

Appendix 6a

Chapter 3: Activity budgets and calculated energy rates

Table A6.i. Summary of results from GLMMs of the effect of troop, season, rank and reproductive state on the activity budget components (% time spent resting, travelling, feeding and in social behaviours). D statistic relates, in the case of the 2-factor null model, to the 1-factor null model and in the case of all other models in this table to the 2-factor null model (continued over page).

Independent variable	Explanatory factors	D	d.f.	p	AIC	VPC	Coefficient category	Coefficient	s.e.	z	p
% time resting	Null (2-factor)	13.22	1	**<0.001**	1034.0	0.19					
	Troop	9.17	1	**0.002**	1026.9	0.07	Gamgam - Kwano =	9.836	2.775	3.55	**<0.001**
	Season	16.68	1	**<0.001**	1019.4	0.20	Wet - Dry =	8.848	2.096	4.22	**<0.001**
	Rank	2.43	2	0.291	1035.6	0.15	Mid - High =	-5.685	4.125	1.38	0.168
							Low - High =	0.816	3.955	0.21	0.837
							Low - Mid =	6.502	4.334	1.50	0.134
	Reproductive state	3.24	2	0.198	1034.8	0.24	Pregnant - Cycling =	7.042	3.972	1.77	***0.076***
							Lactating - Cycling =	4.522	3.17	1.43	0.154
							Lactating - Pregnant =	-2.52	3.731	0.68	0.500
With interaction effects:	Troop+Season	0.43	1	0.514	1011.0	0.06					
	Troop+Rank	1.03	2	0.598	1031.4	0.04					
	Troop+Reproductive state	2.99	2	0.224	1028.2	0.45					
	Season+Rank	0.22	2	0.894	1025.5	0.17					
	Season+Reproductive state	1.70	2	0.428	1025.1	0.22					
	Rank + Reproductive State	2.92	4	0.572	1042.4	0.65					
% time travelling	Null (2-factor)	0.34	1	0.560	889.1	0.02					
	Troop	0.30	1	0.587	890.8	0.02	Gamgam - Kwano =	-0.759	1.372	0.55	0.580
	Season	7.14	1	**0.008**	883.9	0.02	Wet - Dry =	-3.494	1.29	2.71	**0.007**
	Rank	0.40	2	0.818	892.7	0.03	Mid - High =	1.111	1.75	0.64	0.525

Table A6.i. Continued from previous page

Independent variable	Explanatory factors	D	d.f.	p	AIC	VPC	Coefficient category		Coefficient	s.e.	z	p
								Low - High =	0.427	1.634	0.26	0.794
								Low - Mid =	-0.684	1.827	0.37	0.708
	Reproductive state	0.56	2	0.758	892.5	0.01		Pregnant - Cycling =	1.466	1.988	0.74	0.461
								Lactating - Cycling =	-0.02	1.417	0.01	0.989
								Lactating - Pregnant =	-1.486	1.912	0.78	0.437
With interaction effects:	Troop+Season	0.53	1	0.469	886.9	0.02	Effect of Rank within Kwano troop:	Mid - High =	3.635	2.005	1.81	***0.070***
	Troop+Rank	6.14	2	**0.046**	892.3	0.00		Low - High =	3.546	2.127	1.67	***0.096***
	Troop+Reproductive state	4.16	2	0.125	894.1	0.002		Low - Mid =	-0.089	2.237	0.04	0.968
	Season+Rank	0.58	2	0.749	891.2	0.03	Effect of Rank within Gamgam troop:	Mid - High =	-3.202	2.547	1.26	0.202
	Season+Reproductive state	1.66	2	0.437	888.7	0.004		Low - High =	-2.778	1.972	1.41	0.159
	Rank + Reproductive State	1.11	4	0.892	903.0	0.03		Low - Mid =	0.424	2.532	0.17	0.867
% time spent feeding	Null (2-factor)	3.53	1	**0.060**	1046.5	0.09						
	Troop	9.55	1	**0.002**	1038.9	0.005		Gamgam - Kwano =	-8.309	2.288	3.63	**<0.001**
	Season	12.51	1	**<0.001**	1035.9	0.10		Wet - Dry =	-8.266	2.276	3.63	**<0.001**
	Rank	4.29	2	0.117	1046.2	0.04		Mid - High =	4.841	3.333	1.45	0.147
								Low - High =	-2.971	3.135	0.95	0.343
								Low - Mid =	-7.812	3.488	2.24	**0.025**
	Reproductive state	0.92	2	0.632	1049.5	0.10		Pregnant - Cycling =	-2.17	3.999	0.54	0.587
								Lactating - Cycling =	-2.938	3.023	0.97	0.331
								Lactating - Pregnant =	-0.768	3.776	0.20	0.839
With interaction effects:	Troop+Season	0.07	1	0.790	1028.3	0.00						

Table A6.i. Continued from previous page

Independent variable	Explanatory factors	D	d.f.	p	AIC	VPC	Coefficient category		Coefficient	s.e.	z	p
	Troop+Rank	0.04	2	0.980	1042.7	0.00						
	Troop+Reproductive state	3.29	2	0.193	1041.9	0.00						
	Season+Rank	0.11	2	0.948	1040.9	0.07						
	Season+Reproductive state	4.53	2	0.104	1039.1	0.10						
	Rank + Reproductive State	4.70	4	0.320	1053.4	0.05						
% time in social behaviours	Null (2-factor)	6.01	1	**0.014**	873.8	0.14						
	Troop	0.22	1	0.638	875.5	0.61	Gamgam - Kwano =		-0.862	1.824	0.47	0.662
	Season	5.76	1	**0.016**	870.0	0.17	Wet - Dry =		2.906	1.187	2.45	**0.014**
	Rank	0.47	2	0.791	877.3	0.61	Mid - High =		-0.507	2.186	0.23	0.817
							Low - High =		1.024	2.093	0.49	0.625
							Low - Mid =		1.53	2.297	0.67	0.505
	Reproductive state	2.93	2	0.231	874.8	0.12	Pregnant - Cycling =		-2.292	2.062	1.11	0.266
							Lactating - Cycling =		1.09	1.572	0.69	0.488
							Lactating - Pregnant =		3.383	1.945	1.74	***0.082***
With interaction effects:	Troop+Season	0.40	1	0.529	873.5	0.17	Effect of Rank within Kwano troop:	Mid - High =	-3.76	2.072	1.82	***0.070***
	Troop+Rank	6.42	2	**0.040**	880.4	0.03		Low - High =	0.611	2.199	0.28	0.781
	Troop+Reproductive state	0.15	2	0.927	883.8	0.11		Low - Mid =	4.371	2.308	1.89	***0.058***
	Season+Rank	0.66	2	0.720	877.0	0.16	Effect of Rank within Gamgam troop:	Mid - High =	6.424	2.796	2.30	**0.022**
	Season+Reproductive state	1.00	2	0.606	873.0	0.15		Low - High =	2.599	2.205	1.18	0.238
	Rank + Reproductive State	2.80	4	0.593	883.8	0.14		Low - Mid =	-3.825	2.789	1.37	0.170

Table A6.ii. Summary of results from GLMMs of the relationships between calculated energy intake rate ($Log_{10,}$ kJ/hr) and the weather and fruit index variables, plus the effect of troop season, reproductive state and rank on these relationships (continued over page).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2-factor	Null 1-factor	1.62	1	0.203	2205.0	0.04					
Daily min temp	Null 2-factor	19.04	1	**<0.001**	2188.0	0.10		188.13	40.99	4.59	**<0.001**
Daily max temp	Null 2-factor	7.89	1	**0.005**	2199.2	0		137.94	43.92	3.14	**0.002**
Daily rainfall	Null 2-factor	0.68	1	0.408	2206.4						
Month's mean min temp	Null 2-factor	22.42	1	**<0.001**	2184.6	0.08		251.30	50.69	4.96	**<0.001**
Month's mean max temp	Null 2-factor	19.90	1	**<0.001**	2187.1	0		265.93	54.97	4.84	**<0.001**
30 day rainfall	Null 2-factor	2.38	1	0.123	2204.7						
Total-fruit index	Null 2-factor	30.51	1	**<0.001**	2176.5	0.002		0.49	0.08	6.06	**0.009**
Vine-fruit index	Null 2-factor	7.52	1	**0.006**	2199.5	0.09		1.62	0.57	2.83	**0.005**
Tree-fruit index	Null 2-factor	27.08	1	**<0.001**	2180.0	0		0.48	0.08	5.68	**<0.001**
Troop	Null 2-factor	10.79	1	**0.001**	2196.2	0.00	Gamgam-Kwano=	693.093	192.07	3.61	**<0.001**
Season	Null 2-factor	2.75	1	***0.097***	2204.3	0.05	Wet-Dry	343.07	205.73	1.67	***0.095***
Reproductive state	Null 2-factor	4.29	2	0.117	2204.7	0.06	Pregnant-Cycling	637.318	335.016	1.90	0.057
							Lactating-Cycling	0.15	250.335	0.001	0.999
							Lactating-Pregnant	-637.185	317.13	2.01	0.045
Rank	Null 2-factor	2.62	2	0.270	2206.4						
Troop +*											
Daily min temp	Equivalent without interaction	0.01	1	0.924	2174.8						
Daily max temp	Equivalent without interaction	0.35	1	0.557	2196.9						
Daily rainfall	Equivalent without interaction	2.42	1	0.120	2197.8						
Month's mean min temp	Equivalent without interaction	0.04	1	0.838	2175.4						
Month's mean max temp	Equivalent without interaction	1.04	1	0.308	2189.3						
30 day rainfall	Equivalent without interaction	1.27	1	0.261	2198.7						
Total-fruit index	Equivalent without interaction	1.95	1	0.162	2172.4						
Vine-fruit index	Equivalent without interaction	0.06	1	0.800	2189.7						

Table A6.ii. Continued from previous page

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Tree-fruit index	Equivalent without interaction	3.04	1	***0.081***	2175.4		Kwano	0.264	0.122	2.16	**0.030**
							Gamgam	0.562	0.118	4.76	**<0.001**
Season +*											
Daily min temp	Equivalent without interaction	0.47	1	0.492	2191.5						
Daily max temp	Equivalent without interaction	0.11	1	0.736	2198.5						
Daily rainfall	Equivalent without interaction	4.63	1	**0.031**	2200.7	0.04	Dry	133.16	72.95	1.83	***0.068***
							Wet	-28.18	13.25	2.13	**0.033**
Month's mean min temp	Equivalent without interaction	4.53	1	**0.033**	2182.9	0.05	Dry	242.02	62.63	3.86	**<0.001**
	Equivalent without interaction						Wet	716.13	201.03	3.56	**<0.001**
Month's mean max temp	Equivalent without interaction	2.15	1	0.143	2181.2						
30-day rainfall	Equivalent without interaction	8.37	1	**0.004**	2188.5	0	Dry	0.06	1.12	0.05	0.959
							Wet	-4.09	0.85	4.80	**<0.001**
Total-fruit index	Equivalent without interaction	0.01	1	0.933	2180.0						
Vine-fruit index	Equivalent without interaction	2.74	1	***0.098***	2200.5						
Tree-fruit index	Equivalent without interaction	0.76	1	0.384	2183.2	0					
Reproductive state +*											
Daily min temp	Equivalent without interaction	3.34	2	0.189	2190.0	0					
Daily max temp	Equivalent without interaction	4.31	2	0.116	2199.7	0					
Daily rainfall	Equivalent without interaction	2.49	2	0.288	2206.8	0					
Month's mean min temp	Equivalent without interaction	0.94	2	0.624	2189.4						
Month's mean max temp	Equivalent without interaction	10.1	2	0.006	2180.3	0	Cycling	107.886	80.52	1.34	0.180
Month's mean max temp, but with two outliers removed	Equivalent without interaction	4.87	2	0.088			cycling with two outliers removed	229.896	101.849	2.26	0.024
							Pregnant	230.747	119.71	1.93	0.054
							Lactating	485.984	85.473	5.69	0.000
30-day rainfall	Equivalent without interaction	2.04	2	0.360	2206.4						
Total-fruit index	Equivalent without interaction	3.17	2	0.205	2179.6						

Table A6.ii. Continued from previous page

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Vine-fruit index	Equivalent without interaction	2.33	2	0.312	2202.2						
Tree-fruit index	Equivalent without interaction	3.36	2	0.187	2182.6						
Rank +*											
Daily min temp	Equivalent without interaction	2.34	2	0.310	2192.1						
Daily max temp	Equivalent without interaction	1.83	2	0.400	2203.2						
Daily rainfall	Equivalent without interaction	1.10	2	0.576	2210.5						
Month's mean min temp	Equivalent without interaction	1.49	2	0.474	2190.0						
Month's mean max temp	Equivalent without interaction	8.12	2	**0.017**	2184.8	0	High	239.06	81.47	2.93	**0.003**
							Middle	72.61	97.81	0.74	0.458
							Low	479.25	101.78	4.71	**<0.001**
30 day rainfall	Equivalent without interaction	1.95	2	0.376	2207.8						
Total-fruit index	Equivalent without interaction	0.77	2	0.679	2182.9						
Vine-fruit index	Equivalent without interaction	1.07	2	0.585	2204.0						
Tree-fruit index	Equivalent without interaction	0.48	2	0.789	2186.5						
Interactions between categorical variables:											
Troop + Season*	Equivalent without interaction	0.28	1	0.596	2196.0						
Troop + Reproductive state*	Equivalent without interaction	4.17	2	0.124	2194.1						
Troop + Rank*	Equivalent without interaction	1.27	2	0.531	2201.2						
Season + Reproductive state*	Equivalent without interaction	0.57	2	0.752	2208.2						
Season + Rank*	Equivalent without interaction	0.77	2	0.679	2209.4						
Reproductive state + Rank*	Equivalent without interaction	8.27	4	***0.082***	2205.5	See table below for results of z tests from this GLMM					

Table A6.iii. Results of z tests from GLMM of the effect of reproductive state and rank (and their interaction) on calculated energy intake rate ($Log_{10,}$ kJ/hr).

1st reference category	2nd reference category (a)	Comparison category (b)	Coefficient (b-a)	s.e.	z	p
High	Cycling	Pregnant	850.177	409.553	2.08	**0.038**
	Cycling	Lactating	-543.596	327.612	1.66	***0.097***
	Pregnant	Lactating	-1393.773	412.589	3.38	**0.001**
Middle	Cycling	Pregnant	408.251	542.862	0.75	0.452
	Cycling	Lactating	-212.743	423.535	0.50	0.616
	Pregnant	Lactating	-620.995	588.176	1.06	0.291
Low	Cycling	Pregnant	-592.019	657.601	0.90	0.368
	Cycling	Lactating	117.077	424.48	0.28	0.783
	Pregnant	Lactating	709.093	657.601	1.08	0.281
Cycling	High	Middle	-377.105	341.294	1.11	0.269
	High	Low	93.338	443.354	0.21	0.833
	Middle	Low	470.443	456.302	1.03	0.303
Pregnant	High	Middle	-819.031	588.176	1.39	0.164
	High	Low	-1348.856	635.303	2.12	**0.034**
	Middle	Low	-529.826	720.366	0.74	0.462
Lactating	High	Middle	-46.252	412.589	0.11	0.911
	High	Low	754.012	301.578	2.50	**0.012**
	Middle	Low	800.264	389.042	2.06	**0.040**

Table A6.iv. Summary results from GLMMs of the relationships between calculated energy expenditure rate ($Log_{10,}$ kJ/hr) and the weather and fruit index variables, plus the effect of troop season, reproductive state and rank on these relationships (continued over page).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2 level (ID)	Null 1-factor	0.26	1		1484.8	0.02		541.13			
Daily min temp	Null 2-factor	0	1	1	1486.8						
Daily max temp	Null 2-factor	5.76	1	**0.016**	1481.1	0		6.89	2.78	2.48	**0.013**
Daily rainfall	Null 2-factor	9.12	1	**0.003**	1477.7	0		-2.33	0.75	3.12	**0.002**
Month's mean min temp	Null 2-factor	0.01	1	0.92	1486.8						
Month's mean max temp	Null 2-factor	16.73	1	**<0.001**	1470.1	0		14.85	3.49	4.26	**<0.001**
30 day rainfall	Null 2-factor	12.39	1	**<0.001**	1474.4	0		-0.13	0.04	3.69	**<0.001**
Total-fruit index	Null 2-factor	0.25	1	0.617	1486.6						
Tree-fruit index	Null 2-factor	1.46	1	0.228	1485.4						
Vine-fruit index	Null 2-factor	0.50	1	0.479	1486.3						
Troop	Null 2-factor	8.54	1	**0.003**	1478.3	0	Gamgam-Kwano	36.63	12.14	3.02	**0.003**
Season	Null 2-factor	12.59	1	**<0.001**	1474.2	0.02	Wet-Dry	-45.5	12.51	3.64	**<0.001**
Reproductive state	Null 2-factor	7.36	2	**0.025**	1481.5	0.01	Pregnant-Cycling	-47.67	19.3	2.47	**0.014**
							Lactating-Cycling	-29.43	13.8	2.13	**0.033**
							Lactating-pregnant	18.24	18.53	0.98	0.325
Rank	Null 2-factor	0.44	2	0.509	1488.4						
Troop +*											
Daily min temp	Equivalent without interaction	0.03	1	0.854	1482.1						
Daily max temp	Equivalent without interaction	0.72	1	0.398	1480						
Daily rainfall	Equivalent without interaction	2.70	1	0.101	1474		Kwano	-2.67	0.91	2.93	**0.003**
							Gamgam	0.017	1.351	0.01	0.990
Month's mean min temp	Equivalent without interaction	0.26	1	0.610	1482						
Month's mean max temp	Equivalent without interaction	3.22	1	***0.073***	1470.6	0	Kwano	24.84	7.79	3.19	**0.001**
							Gamgam	7.75	5.39	1.44	0.151
30 day rainfall	Equivalent without interaction	0.27	1	0.606	1475.2						
Total-fruit index	Equivalent without interaction	0.24	1	0.623	1482						
Tree-fruit index	Equivalent without interaction	2.61	1	0.106	1479						

Table A6.iv. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Vine-fruit index	Equivalent without interaction	0.06	1	0.808	1482.2						
Season +*											
Daily min temp	Equivalent without interaction	0.20	1	0.655	1475.5						
Daily max temp	Equivalent without interaction	0.57	1	0.449	1473						
Daily rainfall	Equivalent without interaction	1.16	1	0.281	1473.8						
Month's mean min temp	Equivalent without interaction	5.14	1	**0.042**	1468	0	Dry	5.81	4.04	1.44	0.151
							Wet	37.53	12.67	2.96	**0.003**
Month's mean max temp	Equivalent without interaction	1.54	1	0.215	1463.2						
30 day rainfall	Equivalent without interaction	0.20	1	0.653	1474.5						
Total-fruit index	Equivalent without interaction	0.08	1	0.777	1472.4	0	Dry	0.017	0.01	1.74	***0.082***
							Wet	0.013	0.007	1.76	***0.078***
Tree-fruit index	Equivalent without interaction	0.62	1	0.433	1477.6	0.01	Dry	0.04	0.055	0.72	0.472
	Equivalent without interaction						Wet	-0.023	0.055	0.41	0.683
Vine-fruit index	Equivalent without interaction	0.49	1	0.483	1472.2		Dry	0.021	0.011	1.88	***0.060***
							Wet	49.25	17.84	2.76	**0.006**
Reproductive state +*											
Daily min temp	Equivalent without interaction	4	2	0.135	1483.2						
Daily max temp	Equivalent without interaction	0.46	2	0.794	1481.2						
Daily rainfall	Equivalent without interaction	2.12	2	0.346	1478.4						
Month's mean min temp	Equivalent without interaction	7.09	2	**0.029**	1480.2	0	Cycling	2.75	4.4	0.63	0.532
							Pregnant	58.61	22.63	2.59	**0.010**
							Lactating	-4.06	5.52	0.74	0.462
Month's mean max temp	Equivalent without interaction	1.83	2	0.402	1470.9						
30 day rainfall	Equivalent without interaction	3.08	2	0.214	1473.6						
Total-fruit index	Equivalent without interaction	1.73	2	0.422	1484.8						
Tree-fruit index	Equivalent without interaction	1.25	2	0.536	1485.5						
Vine-fruit index	Equivalent without interaction	1.36	2	0.508	1484.9						
Rank state +*											
Daily min temp	Equivalent without interaction	0.96	2	0.619	1493.4						

Table A6.iv. Continued from previous page.

Explanatory factors	Comparison model	D	d.f.	p	AIC	VPC		Coefficient	s.e.	z	p
Daily max temp	Equivalent without interaction	0.47	2	0.791	1487						
Daily rainfall	Equivalent without interaction	3.33	2	0.189	1481.5						
Month's mean min temp	Equivalent without interaction	2.18	2	0.336	1492.2						
Month's mean max temp	Equivalent without interaction	0.13	2	0.936	1476						
30 day rainfall	Equivalent without interaction	1.02	2	0.601	1480.2						
Total-fruit index	Equivalent without interaction	1.27	2	0.531	1492.6						
Tree-fruit index	Equivalent without interaction	4.42	2	0.110	1488.4						
Vine-fruit index	Equivalent without interaction	0.68	2	0.710	1492.7						
Interactions between categorical variables:											
Troop + Season*	Equivalent without interaction	3.46	1	***0.063***	1466.3	See table below for results of z tests from this GLMM					
Troop + Reproductive state*	Equivalent without interaction	0.64	2	0.728	1481						
Troop + Rank*	Equivalent without interaction	2.39	2	0.302	1481.9						
Season + Reproductive state*	Equivalent without interaction	2.74	2	0.254	1474.8						
Season + Rank*	Equivalent without interaction	3.81	2	0.149	1478.4						
Reproductive state + Rank*	Equivalent without interaction	11.81	4	**0.019**	1481.6	See table below for results of z tests from this GLMM					

Table A6.v. Results of z tests from GLMMs of the effect of pairs of categorical variables (troop and season; rank and reproductive state) on calculated energy expenditure rates ($Log_{10,}$ kJ/hr) (continued over page).

1st reference category	2nd reference category (a)	Comparison category (b)	Coefficient (b-a)	s.e.	z	p
Kwano	Dry	Wet	-66.183	16.854	3.93	**<0.001**
Gamgam	Dry	Wet	-21.262	17.094	1.24	0.214
Dry	Kwano	Gamgam	5.23	19.39	0.27	0.787
Wet	Kwano	Gamgam	50.151	14.153	3.54	**<0.001**
High	Cycling	Pregnant	7.20	27.11	0.27	0.790
	Cycling	Lactating	16.01	22.33	0.72	0.473
	Pregnant	Lactating	8.81	26.38	0.33	0.738
Middle	Cycling	Pregnant	-99.45	35.12	2.83	**0.005**

Table A6.v. Continued from previous page.

1st reference category	2nd reference category (a)	Comparison category (b)	Coefficient (b-a)	s.e.	z	p
	Cycling	Lactating	-89.76	28.04	3.20	**0.001**
	Pregnant	Lactating	9.70	38.61	0.25	0.802
Low	Cycling	Pregnant	-94.83	41.58	2.28	**0.023**
	Cycling	Lactating	-42.41	26.77	1.58	0.113
	Pregnant	Lactating	52.43	35.70	1.47	0.142
Cycling	High	Middle	59.88	23.52	2.55	**0.011**
	High	Low	45.62	29.38	1.55	0.120
	Middle	Low	-14.26	30.15	0.47	0.636
Pregnant	High	Middle	-46.78	38.53	1.21	0.225
	High	Low	-56.42	40.53	1.39	0.164
	Middle	Low	-9.64	46.23	0.21	0.834
Lactating	High	Middle	-45.89	27.46	1.67	***0.095***
	High	Low	-12.80	20.55	0.62	0.533
	Middle	Low	33.09	25.95	1.27	0.202

Appendix 6b

Chapter 4: Urinary C-peptide levels

Table A6.vi. Summary of results from GLMMs of the relationships between UCP levels ($Log_{10,}$ ng/mg creatinine) and the weather and fruit index variables, plus the effect of troop season, reproductive state and rank on these relationships (continued over page).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2-factor	Null 1-factor	0	1		86.4	0					
Daily Min Temp	Null 2-factor	0.11	1	0.741	88.3	0		0.0076	0.023	0.33	0.741
Daily Max Temperature	Null 2-factor	0.41	1	0.521	88	0		0.0123	0.0192	0.64	0.522
Daily Rainfall	Null 2-factor	6.17	1	0.013	82.2	0		0.0127	0.00502	2.53	**0.011**
Daily Humidity	Null 2-factor	0.12	1	0.727	88.3	0		-0.0014	0.004	0.35	0.726
Total Rain in last 30 days	Null 2-factor	6.84	1	**0.009**	81.6	0		0.000608	0.000228	2.67	**0.008**
Months Mean Min Temp	Null 2-factor	2.77	1	***0.096***	85.6	0		0.0445	0.0265	1.68	***0.093***
Months Mean Max Temp	Null 2-factor	0.15	1	0.699	88.3	0		0.00807	0.021	0.38	0.701
Months Total Rain	Null 2-factor	13.42	1	**<0.001**	75	0		0.000855	0.000224	3.82	**<0.001**
Months Mean Humidity	Null 2-factor	0.02	1	0.89	88.4	0		0.000652	0.00477	0.14	0.891
Months Fruiting Index	Null 2-factor	0.36	1	0.547	88	0		0.000024	0.000041	0.59	0.559
Tree-Fruit Index	Null 2-factor	7.08	1	**0.008**	81.3	0		0.000579	0.000213	2.72	**0.007**
Vine-Fruit Index	Null 2-factor	0.02	1	0.896	88.4						
Troop	Null 2-factor	0.05	1	0.83	88.4	0	Kwano-Gamgam=	-0.0182	0.0852	0.21	0.831
Season	Null 2-factor	3.48	1	***0.062***	84.9	0	Wet-Dry=	0.156	0.083	1.88	***0.06***
Reproductive State	Null 2-factor	1.94	2	0.379	88.5	0	Cycling-Pregnant=	-0.011	0.136	0.08	0.936
							Cycling-Lactating=	0.115	0.089	1.29	0.197
							Pregnant-Lactating=	0.126	0.137	0.92	0.358
Rank	Null 2-factor	6.44	2	**0.04**	84	0	High-Middle=	0.062	0.0961	0.65	0.519
							High-Low=	0.254	0.1	2.54	**0.011**
							Middle-Low=	0.192	0.104	1.85	***0.065***

Table A6.vi. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Troop +*											
Daily Min Temperature	Equivalent without interaction	0.70	1	0.404	91.6	0					
Daily Max Temperature	Equivalent without interaction	0.10	1	0.757	91.7	0					
Daily Rainfall	Equivalent without interaction	0.20	1	0.656	86	0					
Months Mean Min Temp	Equivalent without interaction	0.14	1	0.712	89.5	0					
Months Mean Max Temp	Equivalent without interaction	0.89	1	0.345	91.2	0					
Months Total Rain	Equivalent without interaction	0.60	1	0.438	78.4	0					
Months Fruiting Index	Equivalent without interaction	0.07	1	0.786	91.9	0					
Tree-Fruit Index	Equivalent without interaction	1.03	1	0.31	84	0					
Vine-Fruit Index	Equivalent without interaction	0.20	1	0.659	92.1	0					
Season +*											
Daily Min Temperature	Equivalent without interaction	0.14	1	0.712	88.7	0					
Daily Max Temperature	Equivalent without interaction	12.90	1	**0**	75.1	0	Dry	0.068	0.022	3.09	**0.002**
							Wet	-4.599	1.19	3.87	**<0.001**
Daily Rainfall	Equivalent without interaction	0.003	1	0.956	85.6	0					
Months Mean Min Temp	Equivalent without interaction	3.47	1	***0.063***	84.6	0	Dry	0.045	0.03	1.50	0.134
							Wet	-0.138	0.093	1.48	0.138
Months Mean Max Temp	Equivalent without interaction	12.10	1	**0.001**	76.2	0	Dry	0.073	0.025	2.92	**0.004**
							Wet	-0.071	0.031	2.29	**0.022**
30 day rainfall	Equivalent without interaction	2.38	1	0.154	82.9	0					
Months Fruiting Index	Equivalent without interaction	12.99	1	**<0.001**	75.9	0	Dry	0.000158	0.000057	2.77	**0.006**
							Wet	-0.000133	0.000053	2.51	**0.012**
Tree-Fruit Index	Equivalent without interaction	2.030	1	0.154	82.3	0					
Vine-Fruit Index	Equivalent without interaction	11.51	1	**0.001**	77.3	0	Dry	0.000186	0.00007	2.66	**0.008**
							Wet	-0.000118	0.00005	2.36	**0.018**
Reproductive state +*											
Daily Min Temperature	Equivalent without interaction	2.45		0.294	91.9	0					
Daily Max Temperature	Equivalent without interaction	0.51		0.775	93.5	0					

Table A6.vi. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Daily Rainfall	Equivalent without interaction	0.01		0.997	88.6	0					
Months Mean Min Temp	Equivalent without interaction	4.05	2	0.132	88.2	0					
Months Mean Max Temp	Equivalent without interaction	1.16	2	0.561	93.2	0					
Months Total Rain	Equivalent without interaction	2.71	2	0.258	79.2	0					
Months Fruiting Index	Equivalent without interaction	0.33	2	0.847	93.8	0					
Tree-Fruit Index	Equivalent without interaction	1.73	2	0.421	86.5	0					
Vine-Fruit Index	Equivalent without interaction	0.67	2	0.714	93.8	0					
Rank+*											
Daily Min Temperature	Equivalent without interaction	2.26	2	0.323	87.4	0					
Daily Max Temperature	Equivalent without interaction	1.94	2	0.379	87.5	0					
Daily Rainfall	Equivalent without interaction	0.34	2	0.845	85	0					
Months Mean Min Temp	Equivalent without interaction	1.21	2	0.546	86.7	0					
Months Mean Max Temp	Equivalent without interaction	1.79	2	0.409	88.1	0					
Months Total Rain	Equivalent without interaction	1.84	2	0.398	76.1	0					
Months Fruiting Index	Equivalent without interaction	0.70	2	0.704	89.1	0					
Tree-Fruit Index	Equivalent without interaction	1.80	2	0.407	81.4	0					
Vine-Fruit Index	Equivalent without interaction	1.03	2	0.597	88.9	0					
Interactions between categorical variables:											
Troop + Season*	Equivalent without interaction	1.31	1	***0.084***	85.8	0	See table below for results of z tests from this GLMM				
Troop + Rank*	Equivalent without interaction	3.01	2	0.222	86.4	0					
Troop + Reproductive state*	Equivalent without interaction	0.49	2	0.783	93.9	0					
Season + Rank*	Equivalent without interaction	0.42	2	0.811	86.7	0					
Season + Reproductive state*	Equivalent without interaction	1.43	2	0.489	90.0	0					
Rank + Reproductive state*	Equivalent without interaction	3.97	4	0.410	90.8	0					

Table A6.vii. Results of z tests from GLMM of the effect of troop and season, and their interaction, on UCP levels ($Log_{10,}$ ng/mg creatinine).

1st reference category	2nd reference category (a)	Comparison category (b)	Coefficient (b-a)	s.e.	z	p
Kwano	Dry	Wet	0.279	0.107	2.607	**0.009**
Gamgam	Dry	Wet	-0.009	0.126	0.071	0.943
Dry	Kwano	Gamgam	0.124	0.122	1.016	0.310
Wet	Kwano	Gamgam	-0.164	0.112	1.464	0.143

Table A6.viii. Summary of results from GLMMs of the relationship between daily UCP levels ($Log_{10,}$ ng/mg creatinine) and daily calculated energy intake and expenditure rates, plus the effect of troop, season, reproductive state and rank on these relationships (continued over page).

Explanatory factors	Comparison model	D	d.f.	p	AIC	VPC		Coefficient	s.e.	z	p
Daily values (n=47)											
Null 2-factor	Null 1-factor	0	1	1	51.8						
Daily energy intake rate	Null 2-factor	0.08	1	0.773	53.7	0		0.0000162	0.0000561	0.29	0.773
Energy intake rate + Troop*	Equivalent without interaction	4.52	1	**0.034**	53.0	0	Kwano	0.000125	0.000073	1.71	***0.087***
							Gamgam	-0.000109	0.000079	1.38	0.168
Energy intake rate + Season*	Equivalent without interaction	1.68	1	0.195							
Energy intake rate + Reproductive state*	Equivalent without interaction	5.73	2	***0.057***	54.0		Cycling	-0.000153	0.000085	1.80	***0.072***
							Pregnant	0.00002	0.000266	0.08	0.940
							lactating	0.000115	0.000068	1.69	***0.091***
Energy intake rate + Rank*	Equivalent without interaction	8.63	2	**0.013**	47.5		High	-0.000208	0.000095	2.19	**0.029**
							Middle	0.000152	0.000071	2.14	**0.032**
							Low	-0.00003	0.000105	0.27	0.775
Daily energy expenditure rate	Null 2-factor	0.43	1	0.513	53.4						
Energy expenditure rate + Troop*	Equivalent without interaction	0.08	1	0.781	57.2						
Energy expenditure rate + Season*	Equivalent without interaction	0	1	1							
Energy expenditure rate + Reproductive state*	Equivalent without interaction	0.63	2	0.731	58.6						
Energy expenditure rate + Rank*	Equivalent without interaction	3.56	2	0.919	52.2						
Monthly values (n=85)											
Null 2-factor	Null 1-factor	0	1	1	86.4	0					

Table A6.viii. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Median monthly energy intake rate	Null 2-factor	0.73	1	0.394	87.7						
Energy intake rate + Troop*	Equivalent without interaction	2.10	1	0.147	89.6						
Energy intake rate + Season*	Equivalent without interaction	9.31	1	**0.002**	77.5	0	Dry	0.000152	0.000097	1.57	0.117
							Wet	-0.000229	0.000073	3.14	**0.002**
Energy intake rate + Reproductive state*	Equivalent without interaction	0.63	2	0.732	93.1						
Energy intake rate + Rank*	Equivalent without interaction	2.16	2	0.340	86.2						
Median monthly energy expenditure rate	Null 2-factor	4.07	1	**0.044**	84.3	0		-0.00207	0.00101	2.05	**0.040**
Energy expenditure rate + Troop*	Equivalent without interaction	4.56	1	**0.033**	83.2	0	Kwano	-0.00371	0.00124	2.99	**0.003**
							Gamgam	0.00198	0.00231	0.86	0.391
Energy expenditure rate + Season*	Equivalent without interaction	2.28	1	0.131	84.8						
Energy expenditure rate + Reproductive state*	Equivalent without interaction	2.91	2	0.234	87.5						
Energy expenditure rate + Rank*	Equivalent without interaction	0.26	2	0.877	85.5						

Appendix 6c

Chapter 5: Glucocorticoid levels

Table A6.ix. Summary of results from GLMMs of the relationships between GC levels ($Log_{10,}$ ng/g dry faeces) and the weather, fruit index and energetic status variables, plus the effect of troop season, reproductive state and rank on these relationships (continued over page).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2 level (ID)		33.96	1	<**0.001**	48.8	0.27					
Daily Min Temp	Null 2-factor	1.21	1	0.271	49.6	0.27					
Daily Max Temperature	Null 2-factor	0.33	1	0.564	50.5	0.26					
Daily Rainfall	Null 2-factor	0.30	1	0.584	50.5	0.27					
Total Rain in last 30-days	Null 2-factor	14.91	1	<**0.001**	35.9	0.24		0.00039	0.0001	3.90	<**0.001**
Months Mean Min Temp	Null 2-factor	1.28	1	0.259	49.6	0.27					
Months Mean Max Temp	Null 2-factor	1.50	1	0.220	49.3	0.24					
Months Fruiting Index	Null 2-factor	17.28	1	<**0.001**	33.6	0.25		-0.000075	0.000018	4.17	<**0.001**
Tree Fruit Index	Null 2-factor	7.24	1	**0.007**	43.6	0.26		0.000206	0.000096	2.15	0.032
Vine Fruit Index	Null 2-factor	23.38	1	<**0.001**	27.5	0.24		-0.00008878	0.000018	4.98	<**0.001**
Monthly energy intake rate	Null 2-factor	4.54	1	**0.033**	46.3	0.24		-0.0000598	0.0000276	2.17	0.030
Monthly energy expenditure rate	Null 2-factor	2.37	1	0.124							
Monthly UCP level	Null 2-factor	1.54	1	0.214	49.3	0.27					
Troop	Null 2-factor	14.03	1	<**0.001**	36.8	0.06	Gamgam-Kwano=	-0.261	0.0495	5.27	<**0.001**
Season	Null 2-factor	0.34	1	0.562	50.5	0.27					
Rank	Null 2-factor	0.97	2	0.616	51.9	0.25					
Reproductive State	Null 2-factor	0.09	2	0.956	52.8	0.27					
Pregnancy stage	Null 2-factor	11.85	3	**0.008**	43.0	0.28	Not Pregnant-P3=	-0.283	0.099	2.86	**0.004**
							P1-P3=	-0.400	0.13	3.08	**0.002**
							P2-P3	-0.341	0.111	3.07	**0.002**
							Not pregnant-P2=	0.058	0.076	0.76	0.445
							P1-P2=	-0.059	0.114	0.52	0.604
							Not pregnant-P1	0.117	0.102	1.15	0.251

Table A6.ix. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Troop +*											
Daily Min Temperature	Equivalent without interaction	0.02	1	0.885	40.3	0.07	Kwano	0.007869	0.0163	0.48	0.629
							Gamgam	0.005112	0.009789	0.52	0.602
Daily Max Temperature	Equivalent without interaction	1.00	1	0.318	39.7	0.06	Kwano	-0.014605	0.014632	1.00	0.318
							Gamgam	0.003400	0.01056	0.32	0.747
Daily Rainfall	Equivalent without interaction	0.03	1	0.858	40.3	0.05	Kwano	0.000855	0.001773	0.48	0.630
							Gamgam	0.001425	0.002675	0.53	0.594
Total Rain in last 30-days	Equivalent without interaction	0.17	1	0.680	27.2	0.06	Kwano	0.000414	0.000147	2.82	**0.005**
							Gamgam	0.000332	0.000134	2.48	**0.013**
Months Mean Min Temp	Equivalent without interaction	0.19	1	0.660	40.0	0.07	Kwano	0.019	0.0269	0.71	0.480
							Gamgam	0.006326	0.011751	0.54	0.591
Months Mean Max Temp	Equivalent without interaction	0.22	1	0.638	40.0	0.06	Kwano	-0.00135	0.0189	0.07	0.943
							Gamgam	-0.012460	0.0141	0.88	0.377
Months Fruiting Index	Equivalent without interaction	6.70	1	**0.010**	16.3	0.04	Kwano	-0.000127	0.000026	4.89	**<0.001**
							Gamgam	-0.000037	0.000022	1.68	***0.093***
Tree Fruit Index	Equivalent without interaction	2.10	1	0.147	31.8	0.05	Kwano	0.0004	0.000138	2.90	**0.004**
							Gamgam	0.000122	0.000131	0.93	0.352
Vine Fruit Index	Equivalent without interaction	8.92	1	**0.003**	7.9	0.04	Kwano	-0.000148	0.000026	5.66	**<0.001**
							Gamgam	-0.0000435	0.0000228	1.91	***0.057***
Monthly energy intake rate	Equivalent without interaction	3.55	1	***0.060***	33.7	0.05	Kwano	-0.000135	0.0000514	2.63	**0.009**
							Gamgam	-0.000020	0.0000325	0.62	0.534
Monthly energy expenditure rate	Equivalent without interaction	7.49	1	**0.006**	29.0	0.07	Kwano	0.00013	0.000574	0.23	0.821
							Gamgam	0.002640	0.000756	3.49	**<0.001**
Monthly UCP	Equivalent without interaction	1.27	1	0.257	38.6	0.08	Kwano	0.005631	0.003989	1.41	0.158
							Gamgam	0.000670	0.00225	0.30	0.766
Troop + Season +*											
Daily Min Temperature	Equivalent without interaction	3.51	3	0.319							
Daily Max Temperature	Equivalent without interaction	2.81	3	0.423							
Daily Rainfall	Equivalent without interaction	2.36	3	0.502							

Table A6.ix. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Total Rain in last 30-days	Equivalent without interaction	17.97	3	**<0.001**	15.2	0.05	See SEASON table below for results of z tests				
Months Mean Min Temp	Equivalent without interaction	3.09	3	0.378							
Months Mean Max Temp	Equivalent without interaction	2.82	3	0.420							
Fruit index	Equivalent without interaction	13.53	3	**0.004**	8.7	0.04	See SEASON table below for results of z tests				
Tree-Fruit Index	Equivalent without interaction	15.27	3	**0.002**	22.5	0.03	See SEASON table below for results of z tests				
Vine-Fruit index	Equivalent without interaction	9.52	3	**0.023**	4.4	0.04	See SEASON table below for results of z tests				
Monthly energy intake rate	Equivalent without interaction	6.21	3	0.102							
Monthly energy expenditure rate	Equivalent without interaction	7.54	3	***0.056***	27.5	0.09	See SEASON table below for results of z tests				
Monthly UCP level	Equivalent without interaction	19.66	3	**<0.001**	25.0	0.10	See SEASON table below for results of z tests				
Troop + Reproductive state +*											
Daily Min Temperature	Equivalent without interaction	1.96	6	0.923							
Daily Max Temperature	Equivalent without interaction	6.87	6	0.333							
Daily Rainfall	Equivalent without interaction	3.39	6	0.758							
Total Rain in last 30-days	Equivalent without interaction	7.28	6	0.296							
Months Mean Min Temp	Equivalent without interaction	8.04	6	0.236							
Months Mean Max Temp	Equivalent without interaction	2.69	6	0.846							
Fruit index	Equivalent without interaction	25.81	6	**<0.001**	2.4	0.15	See REPRODUCTIVE STATE table below for results of z tests				
Tree-Fruit Index	Equivalent without interaction	3.03	6	0.805							
Vine Fruit Index	Equivalent without interaction	22.55	6	**0.001**	-2.7	0.14	See REPRODUCTIVE STATE table below for results of z tests				
Monthly energy intake rate	Equivalent without interaction	19.96	6	**0.003**	25.7	0.12	See REPRODUCTIVE STATE table below for results of z tests				
Monthly energy expenditure rate	Equivalent without interaction	8.88	6	0.181							
Monthly UCP level	Equivalent without interaction	9.38	6	0.154							
Troop + Rank +*											
Daily Min Temperature	Equivalent without interaction	4.99	6	0.545							
Daily Max Temperature	Equivalent without interaction	3.32	6	0.767							
Daily Rainfall	Equivalent without interaction	5.56	6	0.475							
Total Rain in last 30-days	Equivalent without interaction	4.02	6	0.675							
Months Mean Min Temp	Equivalent without interaction	5.99	6	0.424							
Months Mean Max Temp	Equivalent without interaction	3.18	6	0.786							

Table A6.ix. Continued from previous page.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Fruit index	Equivalent without interaction	6.81	6	0.339							
Tree-Fruit Index	Equivalent without interaction	7.17	6	0.305							
Vine Fruit Index	Equivalent without interaction	6.94	6	0.327							
Monthly energy intake rate	Equivalent without interaction	7.22	6	0.301							
Monthly energy expenditure rate	Equivalent without interaction	5.62	6	0.467							
Monthly UCP level	Equivalent without interaction	6.37	6	0.383							
Interactions between categorical variables:											
Troop + Season	Equivalent without interaction	1.67	1	0.196	38.7	0.07					
Troop + Rank	Equivalent without interaction	2.59	2	0.275	41.8	0.0006					
Troop + Reproductive state	Equivalent without interaction	0.88	2	0.645	43.3	0.06					
Troop + Pregnancy stage	Equivalent without interaction	5.95	3	0.114	30.7						
Season + Rank	Equivalent without interaction	0.97	2	0.615	56.5	0.26					
Season + Reproductive state	Equivalent without interaction	0.41	2	0.814	58.0	0.26					
Season + Pregnancy stage	Equivalent without interaction	0.26	3	0.968	50.2	0.28					
Rank + Reproductive State	Equivalent without interaction	3.00	4	0.558	60.8	0.28					
Rank + Pregnancy Stage	Equivalent without interaction	5.83	6	0.443	52.5	0.28					

Table A6.x. Results of z tests showing the effect of troop and season on the relationships between GC levels ($Log_{10,}$ ng/g dry faeces) and the significant weather, fruit index and energetic status variables.

Continuous variable	Season	Kwano coefficient	s.e.	z	p	Gamgam coefficient	s.e.	z	p
30-day Rainfall	Dry	0.000429	0.000203	2.11	**0.035**	-0.00022	0.000249	0.88	0.377
	Wet	0.001268	0.000244	5.20	**0.000**	0.00062	0.000189	3.28	**0.001**
Total-fruit index	Dry	-0.0000700	0.0000357	1.96	**0.050**	0.0000001	0.0000362	0.002	0.998
	Wet	-0.0001667	0.0000295	5.66	**<0.001**	-0.0000967	0.0000273	3.55	**<0.001**
Tree-fruit index	Dry	0.0001779	0.000166	1.07	0.284	-0.000262	0.000166	1.58	0.115
	Wet	0.000897	0.000185	4.85	**<0.001**	0.000457	0.000186	2.46	**0.014**
Vine-fruit index	Dry	-0.0000940	0.0000421	2.24	**0.025**	-0.0000069	0.0000431	0.16	0.872
	Wet	-0.000169	0.0000280	-6.03	**<0.001**	-0.0000823	0.0000257	3.20	**0.001**
Calculated energy expenditure rate	Dry	-0.00113	0.00135	0.84	0.403	0.00241	0.00170	1.42	0.156
	Wet	-0.000251	0.00115	0.22	0.827	0.00328	0.00078	4.20	**<0.001**
UCP level	Dry	0.00112	0.00448	0.25	0.803	-0.00367	0.00237	1.55	0.121
	Wet	0.01925	0.00494	3.90	**<0.001**	0.01445	0.004	3.61	**<0.001**

Table A6.xi. Results of z tests showing the effect of troop and reproductive state on the relationships between GC levels ($Log_{10,}$ ng/g dry faeces) and the significant weather, fruit index and energetic status variables.

Continuous variable		Kwano coefficient	s.e.	z	p	Gamgam coefficient	s.e.	z	p
Total-fruit index	Cycling	-0.0000325	0.0000310	1.04	0.295	0.0000211	0.0000271	0.78	0.436
	Pregnant	-0.000267	0.0000462	5.78	**<0.001**	-0.000214	0.000049	4.37	**<0.001**
	Lactating	-0.00017	0.0000347	4.90	**<0.001**	-0.000116	0.000034	3.41	**0.001**
Vine-fruit index	Cycling	-0.0000505	0.0000332	1.52	0.128	0.0000132	0.0000281	0.47	0.638
	Pregnant	-0.0002677	0.0000454	5.90	**<0.001**	-0.000204	0.0000488	4.18	**<0.001**
	Lactating	-0.000181	0.0000336	5.39	**<0.001**	-0.0001175	0.0000334	3.52	**<0.001**
Calculated energy intake rate	Cycling	-0.0000482	0.0000543	0.89	0.375	0.0000991	0.0000419	2.37	**0.018**
	Pregnant	-0.000329	0.0000776	4.24	**<0.001**	-0.000182	0.0000655	2.78	**0.005**
	Lactating	-0.000254	0.0000674	3.77	**<0.001**	-0.000107	0.0000507	2.11	**0.035**

Table A6.xii. Summary of results from GLMMs of the relationships between GC levels ($Log_{10,}$ ng/g dry faeces) and certain weather, fruit index and energetic status variables, with 30-day rainfall controlled for. D statistic related to comparison of model to equivalent model not containing 30-day rainfall.

Explanatory variables	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Variable coefficient**	**s.e.**	**z**	**p**	**Rain coefficient**	**s.e.**	**z**	**p**
30 day rainfall + Troop + (+ all 2-way interactions)														
Daily Min Temp	0.88	3	0.643	30.3	0.05	Kwano	-0.0154	0.0176	0.88	0.382	0.0004744	0.000163	2.91	**0.004**
						Gamgam	-0.00373	0.0101	0.37	0.712	0.0003499	0.000143	2.44	**0.015**
Daily Max Temperature	2.02	3	0.365	29.2	0.07	Kwano	0.00823	0.0165	0.50	0.618	0.000453	0.00017	2.67	**0.008**
						Gamgam	0.01466	0.0109	1.35	0.179	0.000402	0.000143	2.81	**0.005**
Months Mean Min Temp	0.48	3	0.787	30.7	0.05	Kwano	-0.0148	0.0287	0.52	0.606	0.00045	0.000161	2.80	**0.005**
						Gamgam	-0.0059	0.0124	0.48	0.634	0.00036	0.000146	2.46	**0.014**
Months Mean Max Temp	4.71	3	***0.095***	26.5	0.07	Kwano	0.0499	0.0231	2.16	**0.031**	0.00066	0.000185	3.57	**<0.001**
						Gamgam	0.005	0.0154	0.33	0.745	0.000356	0.000151	2.36	**0.018**
Months Fruiting Index	18.98	3	**<0.001**	12.2	0.05	Kwano	-0.0001145	0.000027	4.29	**<0.001**	0.000247	0.000146	1.69	***0.091***
						Gamgam	-0.0000274	0.000022	1.22	0.221	0.000302	0.000131	2.31	**0.021**
Tree Fruit Index	3.83	3	0.148	27.4	0.05	Kwano	0.000289	0.000149	1.94	***0.052***	0.000286	0.00016	1.79	**0.074**
						Gamgam	-0.0000500	0.000148	0.34	0.735	0.000357	0.000154	2.32	**0.020**
Vine Fruit Index	24.86	3	**<0.001**	6.3	0.05	Kwano	-0.000138	0.000020	6.94	**<0.001**	0.00015	0.000149	1.01	0.312
						Gamgam	-0.0000295	0.000023	1.26	0.207	0.000285	0.000132	2.16	**0.031**
Monthly energy intake/hr	21.17	3	**<0.001**	18.5	0.08	Kwano	0.000011	0.000062	0.18	0.860	0.000993	0.000245	4.05	**<0.001**
						Gamgam	0.000124	0.000052	2.39	**0.017**	0.000918	0.000228	4.03	**<0.001**
Monthly energy expenditure	27.12	3	**<0.001**	7.9	0.06	Kwano	0.0041	0.00122	3.36	**0.001**	0.0046	0.00191	2.41	**0.016**
						Gamgam	0.00464	0.00121	3.84	**<0.001**	0.00438	0.00202	2.17	**0.030**
Monthly UCP level	0.28	3	0.868	30.9	0.07	Kwano	0.0022	0.00415	0.53	0.596	0.000384	0.000157	2.45	**0.014**
						Gamgam	-0.000233	0.00222	0.11	0.916	0.000335	0.000136	2.46	**0.014**
Monthly energy intake rate+ Reproductive state	15.23	5	**0.009**	20.5	0.10	Cycling	-0.0000006	0.000063	0.01	0.992	0.00014	0.000053	2.62	**0.009**
						Pregnant	-0.000199	0.00010	1.93	***0.053***	-0.0000586	0.000096	0.61	0.542
						Lactating	-0.000158	0.000092	1.72	***0.086***	-0.000017	0.000083	0.20	0.838
Monthly energy expenditure rate + Season	26.06	4	**<0.001**	9.4	0.06	Dry	0.00262	0.00186	1.41	0.159	0.00392	0.00217	1.81	***0.071***
						Wet	0.00347	0.00169	2.05	**0.040**	0.00477	0.00146	3.27	**0.001**
Monthly UCP level + Season	19.18	4	**0.001**	13.8	0.06	Dry	-0.00154	0.0045	0.34	0.732	-0.00441	0.00236	1.87	***0.062***
						Wet	0.00699	0.00932	0.75	0.453	0.00413	0.00874	0.47	0.636

Appendix 6d:

Chapter 6: Progesterone levels

Table A6.xiii. Summary of results from GLMMs of the effect of categorical variables (troop, *V. donina* (V.D.) availability, reproductive state and pregnancy stage) on PdG levels ($Log_{10,}$ ng/g dry faeces).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2 level (ID)	Null 1-factor	7.36	1	**0.007**	242.0	0.14					
Troop	Null 2-factor	6.75	1	**0.009**	237.3	0.05	Gamgam-Kwano=	-0.228	0.076	3.00	**0.003**
V.D. availability		121.40	1	**<0.001**	122.6	0.25	Vitex-no vitex=	0.5960	0.0462	12.90	**<0.001**
Reproductive state	Null 2-factor	24.12	2	**<0.001**	221.8	0.08	Pregnant-Cycling=	0.492	0.0963	5.11	**<0.001**
							Lactating-Cycling=	0.108	0.0714	1.51	0.130
							Lactating-Pregnant=	-0.384	0.0932	4.12	**<0.001**
Pregnancy stage	Null 2-factor	25.52	3	**<0.001**	222.5	0.05	P1-not pregnant=	0.289	0.156	1.85	***0.064***
							P2-not pregnant=	0.394	0.113	3.49	**<0.001**
							P3-not pregnant=	0.647	0.147	4.40	**<0.001**
							P2-P1=	0.105	0.182	0.58	0.564
							P3-P1=	0.358	0.205	1.75	***0.081***
							P3-P2=	0.253	0.175	1.45	0.148

Table A6.xiv. Results of likelihood tests from GLMMs built to test whether each of the three categorical variables has a significant effect on PdG level ($Log_{10,}$ ng/g dry faeces) when the effects of the other two categorical variables are controlled for. D relates to comparison with the equivalent model not containing the categorical variable of interest and their interaction effect. * indicates that the interaction effect between the two proceeding variables is included in the model. VPC = 0 for all models.

Explanatory factors	**D**	**d.f.**	**p**	**AIC**	**VPC**	**Relevance of results**
V.D. availability + Reproductive state* + troop (no additional interactions)	5.20	1	**0.023**	103.3	0.04	**Troop** has a significant effect on progesterone levels when the effect of V.D. availability and reproductive state are controlled for.
Reproductive state +Troop * + V.D. availability (no additional interactions)	113.27	1	**<0.001**	103.1	0.03	**V.D. availability** has a significant effect on progesterone levels when the effect of troop and reproductive state are controlled for.
V.D. availability +Troop * + Reproductive state (no additional interactions)	22.01	2	**<0.001**	104.7	0.01	**Reproductive state** has a significant effect on progesterone levels when the effect of troop and V.D. availability are controlled for.

Table A6.xv. Results of likelihood tests from GLMMs built to test whether there are any significant interaction effects between the three categorical variables in their effect on PdG level ($Log_{10,}$ ng/g dry faeces). Full model refers to that containing troop, V.D. availability, reproductive state and all 2-way interactions. D statistic for full model relates to comparison with full model without interactions and all other D values relate to comparison with the full model.

Explanatory factors	**D**	**d.f.**	**p**	**AIC**	**VPC**	**Relevance of results**
Full model	81.53	5	0.196	105.5	0.03	Full model:
Full model except interaction between V.D. availability and Troop	81.57	1	0.838	103.6	0.02	N.S. interaction between is V.D. availability and Troop
Full model except interaction between V.D. availability and Reproductive state	85.02	2	0.174	105.0	0.01	N.S. interaction between V.D. availability and Reproductive state
Full model except interaction between Troop and Reproductive state	85.29	2	0.153	105.3	0.04	N.S. interaction between Troop and Reproductive state

Table A6.xvi. Summary of results from GLMMs of the relationship between PdG level ($Log_{10,}$ ng/g dry faeces)and GC level and effect of troop, V.D. availability and reproductive state on this relationship.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**		**Coefficient**	**s.e.**	**z**	**p**
Null 2-factor	Null 1-factor	7.36	1	**0.007**	242.0	0.14					
GC level	Null 2-factor	51.7	1	<**0.001**	192.3	0.07		0.000175	0.000023	7.61	<**0.001**
GC level + Troop*	Equivalent without interaction	1.32	1	0.251	193.7	0.06	Kwano	0.000141	0.0000329	4.27	<**0.001**
							Gamgam	0.000195	0.0000329	5.93	<**0.001**
							Gamgam-Kwano=	-0.173	0.106	1.63	0.103
GC level + V.D. availability*	Equivalent without interaction	0.23	1	0.630	92.6	0.21	No vitex	0.000120	0.000023	5.22	<**0.001**
							Vitex	0.000103	0.000029	3.55	<**0.001**
							V.D. available-No V.D.=	0.557	0.0821	6.78	<**0.001**
GC level + Reproductive state*	Equivalent without interaction	13.93	2	**0.001**	153.9	0	Cycling	0.000118	0.000027	4.37	<**0.001**
							Pregnant	0.000368	0.000065	5.66	<**0.001**
							Lactating	0.000206	0.000032	6.44	<**0.001**

Table A6.xvii. Summary of results from GLMMs of the effect of troop and V.D. availability on the PdG levels ($Log_{10,}$ ng/g dry faeces) of luteal phase samples, the relationship between PdG and GC levels for luteal phase samples and the effect of troop and V.D. availability on this relationship. Results for full model (containing GC level, troop, V.D. availability and all 2-way interactions) included with and without sample number 37 from individual MMK, Gamgam troop. No luteal phase samples were collected from Kwano troop members during the period when *V. doniana* was available.

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**			**Coefficient**	**s.e.**	**z**	**p**
Null 2-factor	Null 1-factor	0	1	1.000	23.4	0						
V.D. Availability	Null 2-factor	5.97	1	**0.015**	19.4	0	V.D. available- No V.D.=		0.326	0.128	2.55	**0.011**
Troop	Null 2-factor	0.06	1	0.810	25.4	0	Gamgam-Kwano=		-0.029	0.12	0.24	0.809
GC level	Null 2-factor	3.53	1	***0.060***	21.9	0			0.0000682	0.0000354	1.93	***0.054***
GC + Troop *	Equivalent without interaction	4.12	2	0.127	21.8	0		Kwano	-0.0000768	0.0000786	0.98	0.329
		7.65	3	***0.054***				Gamgam	0.000105	0.0000382	2.75	**0.006**
							Gamgam-Kwano=		-0.321	0.2	1.61	0.108
GC + V.D. Availability *	Equivalent without interaction	2.24	1	0.135	13.9	0		No V.D.	0.0000853	0.0000306	2.79	**0.005**
								V.D. available	0.000612	0.000345	1.77	***0.076***
							V.D. available- No V.D.=		-0.0608	0.32	0.19	0.849
GC + Troop + V.D. (and all 2-way interactions)	Equivalent without interaction	7.4	3	***0.060***	12.5	0	No V.D.	Kwano	-0.0000768	0.0000632	1.22	0.224
								Gamgam	0.000121	0.0000313	3.87	**<0.001**
							V.D. available	Gamgam	0.000612	0.000311	1.97	**0.049**
With sample 37 (MMK) removed												
Troop + V.D. (and all interactions)	Equivalent without interaction	17.71	7	**0.013**	12.8	0.11	No V.D.	Kwano	-0.0000814	0.000063	1.29	0.196
								Gamgam	0.000343	0.000155	2.21	**0.027**
							V.D. available	Gamgam	0.000617	0.000308	2.00	**0.045**

Table A6.xviii. Summary of results from GLMMs of the relationship between PdG level ($Log_{10,}$ ng/g dry faeces) and the calculated energy rates, plus the effect of troop, V.D. availability and reproductive state on these relationships (continued over page).

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**	**VPC**			**Coefficient**	**s.e.**	**z**	**p**
Null 2-factor	Null 1-factor	4.18	1	**0.041**	79.4	0.26						
Energy intake rate	Null 2-factor	0.95	1	0.329	80.4	0.24			-0.00004623	0.0000465	1.00	0.320
Energy expenditure rete	Null 2-factor	5.38	1	**0.020**	76.0	0.26			-0.00159	0.000671	2.37	**0.018**
V.D. availability + Energy intake rate*	Equivalent without interaction	0.12	1	0.727	40.4	0.28		No V.D	0.00001055	0.0000395	0.27	0.789
								V.D. available	0.0000431	0.0000794	0.54	0.587
								V.D. available - No V.D.=	0.573	0.1228	4.67	**<0.001**
V.D. availability + Energy expenditure rate*	Equivalent without interaction	0.004	1	0.950	39.4	0.30		No vitex	-0.00063	0.00062	1.02	0.310
								V.D. available	-0.000567	0.000877	0.65	0.518
								V.D. available -No V.D. =	0.541	0.546	1.00	0.322
Troop + Energy intake rate*	Equivalent without interaction	0.26	1	0.611	79.7	0.16						
Troop + Energy expenditure rate*	Equivalent without interaction	3.10	1	***0.078***	73.5	0.22		Kwano	-0.0023	0.000848	2.71	**0.007**
								Gamgam	0.0001127	0.00104	0.11	0.914
								Gamgam-Kwano=	-1.565	0.744	2.10	0.035
V.D. availability + Troop + Energy expenditure rate (+ all 2-way interactions)	Equivalent without V.D. availability interactions	1.94	2	0.379	39.6	0.29	No V.D.	Kwano	-0.00105	0.000735	1.43	0.153
							No V.D.	Gamgam	0.000072	0.000853	0.08	0.933
							V.D. available	Kwano	-0.000537	0.001	0.54	0.591
							V.D. available	Gamgam	0.000585	0.00105	0.56	0.578
Reproductive state + Energy intake rate*	Equivalent without interaction	8.88	2	**0.012**	57.1	0.00		Cycling	0.0000167	0.0000574	0.29	0.771
								Pregnant	-0.0000927	0.0000946	0.98	0.327
								Lactating	-0.000256	0.0000672	3.81	**<0.001**
								Pregnant-Cycling	0.789	0.215	3.67	**<0.001**

Table A6.xviii. Continued from previous page.

Explanatory factors	Comparison model	D	d.f.	p	AIC	VPC			Coefficient	s.e.	z	p
								Lactating-Cycling	0.307	0.147	2.09	**0.037**
								Lactating-pregnant	-0.482	0.211	2.28	**0.022**
V.D. availability + Reproductive state + Energy intake rate (+ all 2-way interactions)	Equivalent without V.D. availability interactions	0.45	3	0.931	15.1	0.0035	No V.D.	Cycling	0.0000535	0.0000416	1.29	0.198
							No V.D.	Pregnant	-0.0000828	0.0000769	1.08	0.281
							No V.D.	Lactating	-0.000143	0.000053	2.70	**0.007**
							V.D. available	Cycling	0.000109	0.0000861	1.27	0.206
							V.D. available	Pregnant	-0.0000277	0.000081	0.34	0.732
							V.D. available	Lactating	-0.0000883	0.0000874	1.01	0.313
Reproductive state + Energy expenditure/hr*	Equivalent without interaction	0.02	2	0.989	67.9	0.05		Cycling	-0.00105	0.001097	0.96	0.339
								Pregnant	-0.00125	0.001396	0.90	0.371
								Lactating	-0.000971	0.00097	1.00	0.317
								Pregnant-Cycling	0.631	0.939	0.67	0.502
								Lactating-Cycling	-0.104	0.804	0.13	0.897
								Lactating-pregnant	-0.735	0.866	0.85	0.396
								Lactating-pregnant	-0.482	0.211	2.28	**0.022**
V.D. availability + Reproductive state + Energy expenditure rate + (+ all 2-way interactions)	Equivalent without V.D. availability interactions	1.48	3	0.224	23.0	0.0783	No V.D.	Cycling	-0.000503	0.000768	0.66	0.512
							No V.D.	Pregnant	-0.000206	0.00104	0.20	0.843
							No V.D.	Lactating	-0.000143	0.000974	0.15	0.883
							V.D. available	Cycling	0.000226	0.00129	0.18	0.861
							V.D. available	Pregnant	0.000522	0.00139	0.38	0.707
							V.D. available	Lactating	0.000585	0.000828	0.71	0.480

Table A6.xix. Results from likelihood ratio tests from GLMMs of the relationship between PdG ($Log_{10,}$ ng/g dry faeces) and UCP levels and the effect of troop, V.D. availability and reproductive state on this relationship.

Explanatory factors	Comparison model	D	d.f.	p	AIC	VPC
Null 2-factor model	Null 1-factor	0	1	1.000	53.7	0
UCP	Null 2-factor	1.53	1	0.217	54.2	0
UCP + Troop*	Equivalent without interaction	1.58	1	0.209	55.1	0
UCP + Vitex *	Equivalent without interaction	0.07	1	0.786	31.8	0.13
UCP + Reproductive state *	Equivalent without interaction	1.59	2	0.451	49.4	0

Table A6.xx. Summary of results from GLMMs of the relationships between individuals' yearling production rates and their median PdG, GC and UCP levels; and calculated energy intake and expenditure rates over the study period. Plus the effect of troop and rank on these relationships (continued over page).

Explanatory factors	Comparison model	D	d.f.	p	AIC		Coefficient	s.e.	z	p
Null model (single factor, ID)					-94.4					
PdG level	Null	0.67	1	0.414	-93.0					
GC level	Null	1.23	1	0.268	-93.6					
UCP level	Null	2.20	1	0.138	-94.6					
Energy balance	Null	0.21	1	0.651	-92.6					
Energy intake rate	Null	0.51	1	0.476	-92.9					
Energy expenditure rate	Null	1.00	1	0.318	-93.4					
Troop	Null	2.08	1	0.150	-94.4					
Troop + PdG level*	Equivalent without interaction	2.39	1	0.123	-94.9	Kwano	0.00000063	0.00000036	1.75	***0.080***
		6.49	3	0.090		Gamgam	-0.00000403	0.00000288	1.40	0.162
Troop + PdG level * (DRK removed)	Equivalent without interaction	-1.38	3	0.711	-87.0	Kwano	0.00000037	0.00000094	0.39	0.694
						Gamgam	-0.00000403	0.00000297	1.36	0.175
Troop + GC level*	Equivalent without interaction	0.20	1	0.658	-90.7					
Troop + UCP level*	Equivalent without interaction	0.27	1	0.605	-92.5					
Troop + Energy intake rate*	Equivalent without interaction	19.29	1	**<0.001**	-110.3	Kwano	-0.00001560	0.00000388	4.02	**<0.001**
						Gamgam	0.00003890	0.00000778	5.00	**<0.001**
Troop + Energy expenditure rate*	Equivalent without interaction	0.08	1	0.776	-90.5					

Table A6.xx. Continued from previous page

Explanatory factors	**Comparison model**	**D**	**d.f.**	**p**	**AIC**		**Coefficient**	**s.e.**	**z**	**p**
Rank	Null	3.11	2	0.211	-93.5					
Rank + PdG level*	Equivalent without interaction	2.68	2	0.262	-90.8					
Rank + GC level*	Equivalent without interaction	0.13	2	0.939	-89.0					
Rank +UCP level *	Equivalent without interaction	0.64	2	0.725	-88.8					
Rank + Energy intake rate*	Equivalent without interaction	4.87	2	***0.088***	-92.5	High	0.00000496	0.00000593	0.84	0.403
						Middle	-0.00001160	0.00000651	1.78	***0.075***
						Low	0.00000785	0.00000549	1.43	0.153
Rank + Energy expenditure rate*	Equivalent without interaction	0.04	2	0.983	-88.8					

REFERENCES

Abbott, D. H., Keverne, E. B., Bercovitch, F. B., Shively, C. A., Mendoza, S. P., Saltzman, W., Snowdon, C. T., Ziegler, T. E., Banjevic, M., Garland, T. and Sapolsky, R. M. (2003). Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior 43: 67-82.

Ahlering, M.A., Millspaugh, J.J., Woods, R.J., Western, D. and Eggert, L.S. (2011). Elevated levels of stress hormones in crop-raiding male elephants. Animal Conservation 14: 124-130.

Alberts, S.C., Altmann, J. and Wilson, M.L. (1996). Mate guarding constrains foraging activity of male baboons. Animal Behaviour 51: 1269–1277.

Albrecht E.D., Nightingale M.S. and Townsley J.D. (1978). Stress-Induced Decreases in the Serum Concentration of Progesterone in the Pregnant Baboon. Journal of Endocrinology 77: 425-426.

Albrecht, E.D., Townsley, J.D., (1976). Serum progesterone in the pregnant baboon (Papio papio). Biology of Reproduction 14: 610–612.

Altmann, J. (1980). Baboon Mothers and Infants. Harvard University Press, Cambridge, Massachusetts.

Altmann, J. and Alberts, S.C., (2003a). Variability in reproductive success viewed from a life-history perspective in baboons. American Journal of Human Biology 15: 401–409.

Altmann, J. and Alberts, S.C. (2003b). Intraspecific variability in fertility and offspring survival in a non-human primate: behavioural control of ecological and social sources. In Wachter, K.W. and Bulatao, R,A. (eds.) Offspring: human fertility behavior in a biodemographic perspective. National Academy Press, Washington, DC, pp. 140–169.

Altmann, J. and Alberts, S.C. (2005). Growth rates in a wild primate population: ecological influences and maternal effects. Behavioural Ecology and Sociobiology 57: 490–501.

Altmann, J. and Altmann, S.A. (1977). Life History of Yellow Baboons: Physical Development, Reproductive Parameters, and Infant Mortality. Primates 18: 315-350.

Altmann. J., Altmann, S. and Hausfater, G. (1988). Determinants of reproductive success in savannah baboons (Papio cynocephalus). In Clutton-Brock, T.H. (ed.) Reproductive success, University of Chicago Press, Chicago, pp. 403–418.

Altmann, J. and Muruthi, P. (1988). Differences in daily life between semiprovisioned and wild-feeding baboons. American Journal of Primatology 15: 213-221.

Altmann, J. and Samuels, A. (1992). Costs of maternal care: infant carrying in baboons. Behavioural Ecology and Sociobiology 29: 391–398.

Altmann, J., Schoeller, S.A., Altmann, P., Muruthi, P. and Sapolsky, R. (1993). Body size and fatness of free-living baboons reflect food availability and activity levels. American Journal of Primatology 30: 149–161.

Altmann, S.A. (1974). Baboon space, time, and energy. American Zoologist 14: 221-248.

Altmann, S.A. (1998). Foraging for survival: yearling baboons in Africa. University of Chicago Press, Chicago.

Altmann, S.A. (1991). Diets of yearling female primates (Papio cynocephalus) predict lifetime fitness. Proceedings of the National Academy of Sciences 88:420-423.

Angelier, F., Clément-Chastel, C., Gabrielsen, g.w., Chastel, O., (2007b) Corticosterone and time–activity budget: An experiment with Black-legged kittiwakes. Hormones and Behavior 52: 482-491.

Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouvé, C., Chastel, O. (2007a). Corticosterone and foraging behavior in a pelagic seabird. Physiological and Biochemical Zoology 80: 283–292.

Armitage, K.B. (1991). Factors affecting corticosteroid concentrations in yellow-bellied marmots. Comparative Biochemistry and Physiology 98:47-54.

Bahnak, B.R., Holland, J.C., Verme, L.J. and Ozoga, J.J. (1979). Seasonal and nutritional effects on serum nitrogen constituents in white-tailed deer. Journal of Wildlife Management 43: 454-460.

Bahr, N.I., Palme, R., Möhle, U., Hodges, J.K. and Heistermann, M. (2000). Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. General and Comparative Endocrinology 117: 427-438.

Bahr, N.I., Pryce, C.R., Döbeli, M., Martin and R.D. (1998). Evidence from urinary cortisol that maternal behavior is related to stress in gorillas. Physiology and Behavior 64:429-437.

Banks, W.A., Altmann, J., Sapolsky, R.M., Phillips-Conroy, J.E. and Morley, J.E. (2003). Serum leptin levels as a marker for a syndrome X-like condition in wild baboons. Journal of Clinical Endocrinology and Metabolism 88: 1234-1240.

Bardi, M., French, J.A., Ramirez, S.M. and Brent L. (2004). The role of the endocrine system in baboon maternal behavior. Biological Psychology 55:724–732.

Bardi, M., Shimizu, K., Barrett, G.M., Borgognini-Tarli, S. and Huffman, M.A. (2003). Peripartum cortisol levels and mother–infant interactions in Japanese macaques. American Journal of Physical Anthropology 120: 298–304.

Barrett, L., Halliday, J. and Henzi, S. P. (2006). The ecology of motherhood: the structuring of lactation costs by chacma baboons. Journal of Animal Ecology 75: 875–886.

Barton, R.A. (1993). Sociospatial mechanisms of feeding competition among female olive baboons Papio anubis. Animal Behaviour 46: 791–802.

Barton, R.A. and Whiten, A. (1993) Feeding competition among female olive baboons Papio anubis. Animal Behaviour 46: 777–789.

Barton, R.A., and Whiten, A. (1994). Reducing complex diets to simple rules: Food selection by olive baboons. Behavioral Ecology and Sociobiology 35: 283-293.

Barton, R.A., Byrne, R.W. and Whiten, A. (1996). Ecology, feeding competition and social structure in baboons. Behavioral Ecology and Sociobiology 38: 321-329.

Barton, R.A., Whiten, A., Byrne, R.W., and English, M. (1993). Chemical composition of baboon plant foods: Implications for the interpretation of intra- and interspecific differences in diet. Folia Primatologica 61: 1-20.

Beehner, J.C. and McCann, C., (2008). Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). Physiology and Behavior 95: 508–514.

Beehner, J.C., Bergman, T.J., Cheney, D.L., Seyfarth, R.M. and Whitten, P.L., (2005). The effect of new alpha males on female stress in free-ranging baboons. Animimal Behaviour 69: 1211–1221.

Behie, A.M., Pavelka, M.S.M. and Chapman, C.A., (2010). Sources of variation in fecal cortisol levels in howler monkeys in Belize. American Journal of Primatology 71: 1–7.

Bentley, G.R. (1999). Aping Our Ancestors: Comparative Aspects of Reproductive Ecology. Evolutionary Anthropology 7: 175-185.

Bentley, G.R., Harrington, A.M. and Ellison, P.T. (1998). Dietary composition and ovarian function among the Lese horticulturalist women of the Ituri Forest, Democratic Republic of Congo. European Journal of Clinical Nutrition 52: 261-270.

Bercovitch, F.B. (1987). Female weight and reproduction in a population of olive baboons (Papio anubis). American Journal of Primatology 12: 189–195.

Berman, C.M. and Schwartz. S. (1988). A nonintrusive method for determining relative body-fat in free-ranging monkeys. Amererican Journal of Primatology 14: 53-64.

Berne, R.B., Levy,M.N., Koeppen, B.M. and Stanton, B.A. (eds.), (1998). Physiology. Mosby, Inc., Missouri.

Boisvenue, C. and Running, S.W., (2006). Impacts of climate change on natural forest productivity- evidence since the middle of the 20th century. Global Change Biology 12: 1–21.

Bonier, F., Martin, P.R., Moore, I.T. and Wingfield, J.C. (2009). Do baseline glucocorticoids predict fitness? Trends in Ecology and Evolution 24: 634–642.

Bonser, A.M. and Garcia-Webb, P. (1984). C-peptide measurement: methods and clinical utility. Critical Reviews in Clinical Laboratory Sciences 19: 297–352.

Borries, C., Sommer, V. and Srivastava, A. (1991). Dominance, age, and reproductive success in free-ranging female Hanuman langurs. International Journal of Primatology 12: 230–257.

Boswell, T., Woods, S.C. and Kenagy, G.J., (1994). Seasonal changes in body mass, insulin, and glucocorticoids of free-living goldenmantled ground squirrels. General and Comparative Endocrinology 96: 339–346.

Brennan, E. J., Else, J. G. and Altmann, J. (1985). Ecology and behaviour of a pest primate: vervet monkeys in a tourist lodge habitat. African Journal of Ecology 23: 35-44.

Brent, L.J.N., Semple, S., Dubuc, C., Heistermann, M., and MacLarnon, A. (2011). Social capital and physiological stress levels in free-ranging adult female rhesus macaques. Physiology and Behavior 102: 76–83.

Bronikowski, A.M. and Altmann, J. (1996). Foraging in variable environment: weather patterns and the behavioural ecology of baboons. Behavioral Ecology and Sociobiology 39: 11–25.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B. (2004). Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Bulger, J. and Hamilton, W.J. (1987). Rank and density correlates of inclusive fitness measures in a natural chacma baboon (Papio ursinus) troop. International Journal of Primatology 8: 635–650.

Busch, D.S. and Hayward L.S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142: 2844–2854.

Calvert, J. J. (1985). Food selection by western gorillas (G. g. gorilla) in relation to food chemistry. Oecologia 65: 236–256.

Campbell, B.C. and Gerald, M.S. (2004). Body composition, age and fertility among free-ranging females rhesus macaques (Macaca mulatta). Journal of Medical Primatology 33: 70-77.

Carnegie, S.D., Dubovick, T., Levash, W., Jolly, A. and Pitts, A. (2003). Female dominance status and fecal corticoids in a cooperative breeder with low reproductive skew: ring-tailed lemurs (Lemur catta). Hormones and Behavior 43: 166–179.

Carnegie, S.D., Fedigan, L.M. and Ziegler, T.E. (2011). Social and Environmental Factors Affecting Fecal Glucocorticoids in Wild, Female White-Faced Capuchins (Cebus capucinus). American Journal of Primatology 73: 1–9.

Cavigelli, SA. (1999). Behavioural patterns associated with faecal cortisol levels in wild, free-ranging female ring-tailed lemurs, Lemur catta. Animal Behaviour 57:935–944.

Chapman, C. A., Chapman, L. J., Rode, K. D., Hauck, E. M., and McDowell, L. R. (2003).Variation in the nutritional value of primate foods: Among trees, time periods, and areas. International Journal of Primatology 24: 317–333.

Chapman, C.A. and Chapman, L.J. (2002). Foraging challenges of red colobus monkeys: influence of nutrients and secondary compounds. Comparative Biochemistry and Physiology 133: 861-875.

Chapman, C. A. and Chapman, L. J., Zanne, A. E., Poulsen, J. R., and Clark, C. J. (2005). A 12-year phenological record of fruiting: Implications for frugivore populations and indicators of climate change. In Dew, J.L. and Boubli, J.P. (eds.), Tropical fruits and frugivores, Springer, The Netherlands, pp. 75–92.

Chapman, C.A., Saj, T. and Snaith, T.V. (2007). Temporal dynamics of nutrition, parasitism and stress in colobus monkeys: implications for population regulation and conservation. American Journal of Physical Anthropology 134: 240–250.

Chapman, C.A., Speirs, M.L., Gillespie, T.R., Holland, T. and Austad, K.M. (2006). Life on the edge: gastrointestinal parasites from the forest edge and interior primate groups. American Journal of Primatology 68: 397–409.

Charles-Dominique, P. (1977). Ecology and Behavior of Nocturnal Primates : prosimians of Equatorial West Africa. Gerald Duckworth & Co Ltd., London.

Charnov, E.L., Berrigan, D., (1993). Why do female primates have such long lifespans and so few babies? Or life in the slow lane. Evolutionary Anthropology 6: 191–194.

Cheney, D.L., Seyfarth, R.M., Fischer, J., Beehner, J., Bergman, T., Johnson, S.E., Kitchen, D.M., Palombit, R.A., Rendall, D. and Silk, J.B. (2004). Factors Affecting

Reproduction and Mortality among Baboons in the Okavango Delta, Botswana. International Journal of Primatology 25: 401-428.

Chevallier, A. (2001). Encyclopedia of Medicinal Plants. Dorling Kindersley, London.

Chavez, A.O., Lopez-Alvarenga, J.C., Tejero, M.E., Triplitt, C., Bastarrachea, R.A., Sriwijitkamol, A., Tantiwong, P., Voruganti, V.S., Musi, N., Comuzzie, A.G., DeFronzo, R.A. and Folli, F. (2008). Physiological and Molecular Determinants of Insulin Action in the Baboon. Diabetes 57: 899-908.

Cleland, J.G. and Sathar, Z.A. (1984) The Effect of Birth Spacing on Childhood Mortality in Pakistan. Population Studies 38: 401-418.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J.C. and Smith, J.M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. Proceedings of the Royal Society of London, Series B 271: 2473–2479.

Clutton-Brock, T.H. (1977). Primate Ecology: Studies of Feeding and Ranging Behavior in Lemurs, Monkeys and Apes, Academic Press, New York.

Collier, G. and O'Dea, K. (1983). The effect of coingestion of fat on the glucose, insulin and gastric inhibitory polypeptide responses to carbohydrate and protein. American Journal of Clinical Nutrition 37: 94l-944.

Conklin-Brittain, N.L., Knott, C.D. and Wrangham, R.W., (2006). Energy intake by wild chimpanzees and orangutans: methodological considerations and a preliminary comparison. In Hohmann, G., Robbins, M.M. and Boesch, C. (eds.), Feeding Ecology in Apes and Other Primates, Cambridge University Press, Cambridge, pp. 445–471.

Conklin-Brittain, N.L., Wrangham, R.W. and Hunt, K.D. (1998). Dietary Response of Chimpanzees and Cercopithecines to Seasonal Variation in Fruit Abundance. II. Macronutrients. International Journal of Primatology 19: 971-998.

Cook, B. and Beastall, G.H. (1987). Measurement of steroid hormone concentrations in blood, urine, and tissues. In Green, B. and Leake, R.E. (eds.), Steroid hormones: a practical approach, IRL Press, Oxford, p 1–65.

Cook, R. C., Murray, D. L., Cook, J. G., Zager, P. and Monfort, S. L. (2001). Nutritional influences on breeding dynamics in elk. Canadian Journal of Zoology 79: 845-853.

Cooper, M.A., Chaitra, M.S. and Singh, M. (2004) Effect of dominance, reproductive state, and group size on body mass in Macaca radiata. International Journal of Primatology 25: 165–178.

Cords, M. (1986) Interspecifc and intraspecifc variation in diet of two forest guenons, Cercopithecus ascanius and C. mitis. Journal of Animal Ecology 55: 811-827.

Cowlishaw, G., and Dunbar, R.I.M. (2000) Primate Conservation Biology. University of Chicago Press, Chicago.

Creel, S. (2001). Social dominance and stress hormones. Trends in Ecology and Evolution 16: 491-497.

Creel, S.F., (2005). Dominance, aggression, and glucocorticoid levels in social carnivores. Journal of Mammalogy 86: 255–264.

Crockford, C., Wittig, R.W., Whitten, P.L., Seyfarth, R.M. and Cheney, D.L. (2008). Social stressors and coping mechanisms in wild female baboons (Papio hamadryas ursinus). Hormones and Behavior 53: 254–265.

Dallman, M.F., Strack, A.M., Akana, S.F., Bradbury, M.J., Hanson, E.S., Scribner, K.A. and Smith, M. (1993). Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Frontiers in Neuroendocrinology 14: 303–347.

Dallman, M.F., Warne, J.P., Foster, M.T. and Pecoraro, N.C. (2007). Glucocorticoids and insulin both modulate caloric intake through actions on the brain. Journal of Physiology 583: 431–436.

Dasilva, G.L. (1992). The western black-and-white colobus as a low energy strategist: activity budgets, energy expenditure and energy intake. Journal of Animal Ecology 62: 79-91.

Deschner, T., Kratzsch, J. and Hohmann, G. (2008). Urinary C-peptide as a method for monitoring body mass changes in captive bonobos (Pan paniscus). Hormones and Behavior 54: 620-626.

DeVries, A.C., Glasper, E.R., and Detillion, C.E. (2003). Social modulation of stress responses. Physiology and Behavior 79: 399– 407.

Dittus, W.P.J. (1998). Birth sex ratios in toque macaques and other mammals: integrating the effects of maternal condition and competition Behavioural Ecology and Sociobiology 44: 149-160.

Donaldson, L.E., J.M. Bassett and Thothurn, G.D. (1970). Peripheral plasma progesterone concentration of cows during puberty, oestrus cycles, pregnancy and lactation, and the effects of undernutrition or exogenous oxytocin on progesterone concentration. Journal of Endocrinology 48: 599-614.

Doran, D.M. (1997). Influence of seasonality on activity patterns, feeding behavior, ranging, and grouping patterns in Taï chimpanzees. International Journal of Primatology 18: 183–206.

Doucet, E., St-Pierre, S., Almeras, N., Mauriege, P., Despres, J.P., Richard, D., Bouchard, C., and Tremblay, A. (2000). Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: evidence from both a cross-sectional and an intervention study. Journal of Clinical Endocrinology and Metabolism 85:4231–4237.

Drickamer, L.C. (1974). A ten-year summary of reproductive data for free-ranging Macaca mulatta. Folia Primatologica 21: 61-80.

Dunbar, R.I.M. (1988). Primate Social Systems. Croom Helm, London.

Dunbar, R.I.M. (1992). Time: a hidden constraint on the behavioural ecology of baboons. Behavioural Ecology and Sociobiology 31: 35-49.

Dunbar, R.I.M. and Dunbar, P. (1988). Maternal time budgets of gelada baboons. Animal Behaviour 36: 970-980.

Dunn, A. (1998). Gashaka Gumpti National Park: A Management Plan for Developing the Park and its Support Zone. NPS, NCF and WWF-UK.

Dunn, T.G., Rone, J., Kaltenbach, C.C., van der Walt, L.A., Riley, M.L. and Akbar, A.M. (1974). Hormone changes during underfeeding of beef females. Journal of Animal Science 39: 206. [Abstract]

Egan, H., Ronald, S.K., Pearson, D. and Sawyer, R. (1981) Pearson's chemical analysis of foods, Churchill Livingstone, Edinburgh.

Eley, R.M., Strum, S.C. and Muchemi, G. (1986). Nutritional effects upon physical condition of three free-ranging troops of baboons in Gilgil, Kenya. Primate Report 14: 136.

Eley, R.M., Strum, S.C., Muchemi, G. and Reid, G.D.F. (1989). Nutrition, body condition, activity patterns, and parasitism of free-ranging troops of olive baboons (Papio anubis) in Kenya. American Journal of Primatology 18: 209-220.

Ellison, P.T. (1990). Human ovarian function and reproductive ecology: new hypotheses. American Anthropology 92: 933-952.

Ellison, P.T. (2003). Energetics and Reproductive Effort. American Journal of Human Biology 15: 342–351.

Ellison, P.T. and Valeggia, C.R. (2003). C-Peptide levels and the duration of lactational amenorrhea. Fertility and Sterility 80: 1279-1280.

Emery Thompson, M. (2005). Reproductive endocrinology of wild female chimpanzees (Pan troglodytes schweinfurthii): methodological considerations and the role of hormones in sex and conception. American Journal of Primatology 67: 137-158.

Emery Thompson, M., Kahlenberg, S.M., Gilby, I.C. and Wrangham, R.W. (2007). Core area quality is associated with variance in reproductive success among female chimpanzees at Kibale National Park. Animal Behaviour 73: 501-512.

Emery Thompson, M. and Knott, C.D. (2008). Urinary C-peptide of insulin as a non-invasive marker of energy balance in wild orangutans. Hormones and Behavior 53: 526-535.

Emery Thompson, M., Muller, M.N., Kahlenberg, S.M. and Wrangham, R.W. (2010). Dynamics of ecological and social stress in wild chimpanzees. Hormones and Behavior 58: 440-449.

Emery Thompson, M., Muller, M.N., Wrangham, R.W., Lwanga, J.S. and Potts, K. (2009). Urinary C-peptide tracks seasonal and individual variation in energy balance in wild chimpanzees. Hormones and Behavior 55: 299-305.

Emery Thompson, M., Wilson, M.L., Gobbo, G., MN Muller, and Pusey, A.E. (2008). Hyperprogesteronemia in response to Vitex fischeri consumption in wild chimpanzees (Pan troglodytes schweinfurthii). American Journal of Primatology 70: 1064-1071.

Emery Thompson, M. and Wrangham, R.W. (2008). Diet and Reproductive Function in Wild Female Chimpanzees (Pan troglodytes schweinfurthii) at Kibale Naitonal Park, Uganda. American Journal of Physical Anthropology 135: 171-181.

Engh, A.L., Beehner, J.C., Bergman, T.J., Whitten, P.L., Hoffmeier, R.R., Seyfarth, R.M. and Cheney, D.L., (2006). Female hierarchy instability, male immigration and infanticide increase glucocorticoid levels in female chacma baboons. Animal Behaviour 71: 1227–1237.

Espinal, J. (1989). Understanding Insulin Action: Principles and molecular mechanisms, Ellis Horwood Limited, Chichester.

Fa, J. E. (1991). Provisioning of Barbary Macaques on the Rock of Gibraltar. In Box, H.O.(ed.), Primate Responses to Environmental Change, Chapman & Hall, London. pp. 137-154.

Fajer, A.B., Holzbauer, M. and Newport, H.M. (1971). The contribution of the adrenal gland to the total amount of progesterone produced in the female rat. Journal of Physiology 214: 115-126.

Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D. and Harper, C.W. (2008). Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. Global Change Biology 14: 1600–1608.

Fietz, J. and Ganzhorn, J.U. (1999). Feeding ecology of the hibernating primate Cheirogaleus medius: how does it get so fat? Oecologia 121:157-164.

Fleming, A.S., Steiner, M. and Anderson, V. (1987). Hormonal and attitudinal correlates of maternal behaviour during the early postpartum period in first-time mothers. Journal of Reproductive and Infant Psychology 5: 193–205.

Fleming, A.S., Steiner, M. and Corter, C. (1997). Cortisol, hedonics, and maternal responsiveness in human mothers. Hormones and Behavior 32: 85–98.

Foerster, S. and Monfort, S. (2010). Fecal glucocorticoids as indicators of metabolic stress in female Sykes' monkeys (Cercopithecus mitis albogularis) Hormones and Behavior 58: 685–697.

Foley, C.A.H., Papageorge, S. and Wasser, S.K. (2001). Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15: 1134–1142.

Forthman-Quick, D. L. (1986). Activity budgets and the consumption of human foods in two troops of baboons (Papio anubis) at Gilgil, Kenya. In Else, J. G. & Lee, P. C. (eds.), Primate Ecology and Conservation, Cambridge University Press, New York pp. 221-228.

Forthman-Quick, D.L. and Demment, M.W. (1988). Dynamics of exploitation: differential energetic adaptations of two troops of baboons to recent human contact. In Fa, J.E. and Southwick, C.H. (eds.), Ecology and Behavior of Foodenhanced Primate Groups, Alan R Liss Inc., New York. p 25–52.

Freeland, D. and Janzen, D.H. (1974). Strategies of herbivory in animals: The role of plant secondary compounds. American Naturalist 108: 269-289.

Freeland, W.J. (1976). Pathogens and the evolution of primate sociality. Biotropica 8: 12–24.

French, J.A., Koban, T., Rukstalis, M., Ramirez, S.M., Bardi, M. and Brent, L. (2004). Excretion of urinary steroids in pre- and postpartum female baboons. General Comparative Endocrinology 137: 69–77.

Ganzhorn, J.U. (1992). Leaf chemistry and the biomass of folivorous primates in tropical forests: test of a hypothesis. Oecologia 91: 540–547.

Garcia, C., Lee, P.C. and Rosetta, L. (2006). Dominance and Reproductive Rates in Captive Female Olive Baboons, Papio anubis. American Journal of Physical Anthropology 131: 64-72.

Garcia, C., Lee, P.C., Rosetta, L. (2009). Growth in colony living Anubis baboon infants and its relationship with maternal activity budgets and reproductive status. American Journal of Physical Anthropology 138: 123–135.

Gaulin, S.J.C. (1979). A Jarman/Bell model of primate feeding niches. Human Ecology 7: 1-20.

Gautier-Hion, A. (1980) Seasonal variation of diet related to species and sex in a community of Cercopithecus monkeys. Journal of Animal Ecology 49: 237-269.

Georgiev, A., Emery Thompson, M., Lokasola, A. and Wrangham, R. (2011). Seed predation by bonobos (Pan paniscus) at Kokolopori, Democratic Republic of the Congo. Primates 52: 309-314.

Gesquiere, L., Khan, M., Shek, L., Wango, T., Wango, E., Alberts, S. and Altmann, J. (2008). Coping with a challenging environment: effects of seasonal variability and reproductive status on glucocorticoid concentrations of female baboons (Papio cynocephalus). Hormones and Behavior 54: 410–416.

Girard, I. and Garland, T. (2002) Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. Journal of Applied Physiology 92: 1553–1561.

Girard-Buttoz, C., Higham, J.P., Heistermann, M., Wedegärtner, S., Maestripieri, D. and Engelhardt, A. (2011). Urinary C-peptide measurement as a maker of nutritional status in macaques. PLoS One 6: 1–9.

Goetz, F.C., Roel, J., Jacobs, D.R. Jr., Barbosa, J., Hannan, P., Palmer, J. and Hagopian,W. (2002). Declining beta-cell function in type 2 diabetes: 5-year follow-up and immunologic studies of the population of Wadena, MN. Metabolism 51: 144-148.

Gombe, S., and Hansel, W. (1973). Plasma LH and progesterone levels in heifers on restricted energy intakes. Journal of Animal Science 37: 728–733.

Goymann, W., East, M. L., Wachter, B., Höner, O. P., Möstl, E., Van't Hof, T. J. and Hofer, H. (2001). Social, state-dependent and environmental modulation of faecal glucocorticoid levels in freeranging female spotted hyenas. Proceedings of the Royal Society of London, Series B 268: 2453-2459.

Goymann, W. and Wingfield, J.C. (2004). Allostatic load, social status and stress hormones: the costs of social status matter. Animal Behaviour 67: 591–602.

Gust, D.A., Gordon, T.P., Hambright, K. and Wilson, M.E. (1993). Relationship between social factors and pituitary-adrenocortical activity in female rhesus monkeys (Macaca mulatta). Hormones and Behavior 27:318–31.

Hahn, N.E., Proulx, D., Muruthi, P.M., Alberts, S. and Altmann, J. (2003). Gastrointestinal Parasites in Free-ranging Kenyan Baboons (Papio cynocephalus and P. anubis). International Journal of Primatology 24: 271-279.

Hardy, R.N. (1981). Endocrine physiology. University Park Press, Baltimore MD, USA.

Harris, R.B., Zhou, J., Youngblood, B.D., Rybkin, I.I., Smagin, G.N. and Ryan, D.H. (1998) Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. American Journal of Physiology 275: 1928–1938.

Harris, T.R., Chapman, C.A. and Monfort, S.L. (2009). Small folivorous primate groups exhibit behavioral and physiological effects of food scarcity. Behavioural Ecology and Sociobiology 21: 46–56.

Hayashi, K. and Moberg, G. (1990) Influence of the HPA axis on the menstrual cycle and the pituitary responsiveness to estradiol in the female rhesus monkey. Biology of Reproduction 42: 260–265.

Heistermann, M., Möhle, U., Vervaecke, H., van Elsacker, L. and Hodges, J.K. (1996). Application of urinary and fecal steroid measurements for monitoring ovarian

function and pregnancy in the bonobo (*Pan paniscus*) and evaluation of perineal swelling patterns in relation to endocrine events. *Biology of Reproduction* 55: 844-853.

Henzi, S.P. and Barrett, L. (2003). Evolutionary ecology, sexual conflict, and behavioural differentiation among baboon populations. Evolutionary Anthropology 12: 217-230.

Henzi, S.P. and Barrett, L. (2005). The historical socioecology of savanna baboons (Papio hamadryas). Journal of Zoology 265: 215–226.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C. and Cullinan, W.E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers of Neuroendocrinology 24: 151–180.

Higham, J.P. (2006) The reproductive ecology of female olive baboons (Papio hamadryas anubis) at Gashaka-Gumpti National Park, Nigeria. PhD Thesis, Roehampton University.

Higham, J.P., Girard-Buttoz, C., Engelhardt, A. and Heistermann, M. (2011). Urinary C-Peptide of Insulin as a Non-Invasive Marker of Nutritional Status: Some Practicalities. PLoS ONE 6(7): e22398.

Higham, J.P., Heistermann, M. and Maestripieri, D. (2011). The energetics of male-male endurance rivalry in rhesus macaques. Animal Behaviour 81: 1001-1007.

Higham, J.P., MacLarnon, A.M., Heistermann, M., Ross, C. and Semple, S. (2009b). Rates of self-directed behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (Papio hamadryas anubis). Stress 12: 526-532.

Higham, J.P., Ross, C., Warren, Y., Heistermann, M. and MacLarnon, A.M. (2007). Reduced reproductive function in wild baboons (Papio hamadryas anubis) related to natural consumption of the African black plum (Vitex doniana). Hormones and Behavior 52: 384-390.

Higham, J.P., Warren, Y., Adanu, J., Umaru, B.N., MacLarnon, A.M., Sommer, V. and Ross, C. (2009a). Living on the edge: life-history of olive baboons at Gashaka-Gumti National Park, Nigeria. American Journal of Primatology 71: 293–304.

Hill, C.M. (1997). Crop-raiding by wild vertebrates: The farmer's perspective in an agricultural community in western Uganda. International Journal of Pest Management 43: 77–84.

Hill, D. A. (1999). Effects of provisioning on the social behaviour of Japanese and rhesus macaques: implications for socioeclogy. Primates 40: 187-198.

Hill, R.A., (2006). Thermal constraints on activity scheduling and habitat choice in baboons. American Journal of Physical Anthropology 129: 242–249.

Hill, R.A. and Dunbar, R.I.M. (2002). Climatic determinants of diet and foraging behaviour in baboons. Evolutionary Ecology 16: 579-593.

Hill R.A., Lycett, J.E. and Dunbar, R.I.M. (2000). Ecological and social determinants of birth intervals in baboons. Behavioural Ecology. 11: 560-564.

Hladik, C.M. and Simmen, B. (1998). Taste Perception and Feeding Behavior in Nonhuman Primates and Human populations. Evolutionary Anthropology 5: 58-71.

Hodges, J.K. and Green, D.I. (1989). The development of an enzyme-immunoassay for urinary pregnanediol-3-glucuronide and its application to reproductive assessment in exotic mammals. Journal of Zoology 219: 89-99.

Hodges, J.K. and Heistermann, M. (2003). Field endocrinology: Monitoring hormonal changes in free-ranging primates. In Setchell, J. and Curtis, D. (eds.), Field and laboratory methods in primatology, Cambridge University Press, Cambridge, pp. 282–294.

Hoffman, C.L., Ayala, J.E., Mas-Rivera, A. and Maestripieri D. (2010). Effects of reproductive condition and dominance rank on cortisol responsiveness to stress in free-ranging female rhesus macaques. American Journal of Primatology 72: 559–65.

Hoffman, C.L., Higham, J.P., Heistermann, M., Coe, C.L., Prendergast, B.J. and Maestripieri, D. (2011). Immune function and HPA axis activity in free-ranging rhesus macaques. Physiology and Behavior 104: 507-514.

Holt, R.I.G. and Hanley, N.A. (2010). Essential endocrinology and diabetes, 5th edition. Wiley-Blackwell, MA, USA.

Horwitz, D. L., J. I. Starr, M. E. Mako, W. G. Blackard, and Rubenstein, A. H. (1975). Proinsulin, insulin and C-peptide concentrations in human portal and peripheral blood. Journal of Clinical Investigation 55: 1278-1283.

Hrdy, S.B. (1999). Mother Nature: A History of Mothers, Infants, and Natural Selection, Pantheon Books, New York, NY.

Huber, S., Palme, R. and Arnold, W. (2003). Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (Cervus elaphus). General Comparative Endocrinology 130: 48–54.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M. and Yoshida, K. (1997) Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. Primates 38: 111-125.

IBL International (2009). C-peptide ELISA, Instructions for Use. Hamburg, Germany.

Ido, Y., Vindigni, A., Chang, K., Stramm, L., Chance, R., Heath, W., DiMarchi, R., Cera ,E.D. and Williamson, J. (1997) Prevention of vascular and neural dysfunction in diabetic rats by C-peptide. Science 277: 563–566.

Imakawa, K., Kittok, R.J., and Kinder, J.E. (1983). Influence of dietary energy intake on progesterone concentrations in the beef heifer. Journal of Animal Science 56: 454–459.

Isbell, L.A., Pruetz, J.P., Nzuma, B.M. and Young, T.P. (1999). Comparing measures of travel distances in primates: methodological considerations and socioecological implications. American Journal of Primatology 48: 87–98.

Isbell, L,A, and Young, T.P. (1993). Social and ecological influences on activity budgets of vervet monkeys, and their implications for group living. Behavioural Ecology and Sociobiology 32: 377–385.

Jenni-Eiermann, S., Glaus, E., Grüebler, M., Schwabl, H. and Jenni, H. (2008). Glucocorticoid response to food availability in breeding barn swallows (Hirundo rustica). General and Comparative Endocrinology 155: 558–565.

Johnson, S.E. (2003). Life history and the competitive environment: trajectories of growth, maturation, and reproductive output among Chacma baboons. American Journal of Physical Anthropology 120: 83-98.

Johnson, M. H. and Everitt, B. (2000). Essential Reproduction. Academic Press, New York.

Johnson, E.O., Kamilaris, T.C., Carter, S., Gold, P.W. and Chrousos, G.P. (1991). Environmental-stress and reproductive success in the common marmoset (Callithrix jaccus jaccus). American Journal of Primatology 25: 191–201.

Johnson, R.L and Kapsalis, E. (1995). Ageing, infecundity and reproductive senescence in free-ranging female rhesus macaques. Journal of Reproduction and Fertility 105: 271–278.

Jolly, C. J. (2001). A proper study for mankind: analogies from the papionin monkeys and their implications for human evolution. Yearbook of Physical Anthropology 44: 177–204.

Jolly, C.J. (2007). Baboons, mandrills, and mangabeys: Afro-Papionin socioecology in a phylogenetic perspective. In Campbell, C.J., Fuentes, A., MacKinnon, K.C. and Panger, M. (eds.), Primates in Perspective, Oxford University Press, Oxford, pp. 240–252.

Kappeler, P.M. and Watts, D.P. (eds.) 2011. Long-Term Field Studies of Primates. Springer, Heidelberg.

Kaur, T., and Huffman, M. A. (2004). Descriptive urological record of chimpanzees (Pan troglodytes) in the wild and limitations associated with using multi-reagent dipstick test strips. Journal of Medical Primatology 33: 187–196.

Kemnitz, J.W., Roecker, E.B., Weindruch, R., Elson, D.F., Baum, S.T. and Bergman, R.N. (1994). Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. American Journal of Physiology 266: E540–E547.

Kemnitz, J.W., Sapolsky, R.M., Altmann, J., Muruthi, P., Mott, G.E. and Stefanick, M.L., (2002). Effects of food availability on serum insulin and lipid concentrations in free-ranging baboons. American Journal of Primatology 57: 13–19.

Kenagy, G.J., and N.J. Place. (2000). Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: effects of reproduction and capture and handling. General and Comparative Endocrinology 117: 189–199.

Kenagy, G.J., Place, N.J., Veloso, C., (1999). Relation of glucocorticosteroids and testosterone to the annual cycle of free-living degus in semiarid Chile. General Comparative Endocrinology 115: 236–243.

Key, C.A. and Ross, C. (1999). Sex differences in energy expenditure in non-human primates. Proceedings of the Royal Society London, Series B 266: 2479–2485.

King, A.J. and Cowlishaw, G. (2009). All together now: behavioural synchrony in baboons. Animal Behaviour 78: 1381–1387.

Kingdon, J. (1997). The Kingdon field guide to African mammals. New York: Academic Press.

Kikusui, T., Winslow, J.T. and Mori, Y. (2006). Social buffering: relief from stress and anxiety. Philosophical Transactions of the Royal Society London, Series B 361: 2215–2228.

Kitaysky, A.S., Wingfield, J.C. and Piatt, J.F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Functional Ecology 13: 577–584.

Kjems, L.L., Christiansen, E., Volund, A., Bergman, R.N. and Madsbad, S. (2000). Validation of methods for measurement of insulin secretion in humans in vivo. Diabetes 49: 580–588.

Kleiber, M. (1961). The Fire of Life: An Introduction to Animal Energetics. Wiley, New York.

Knott, C. (1997). Interactions between energy balance, hormonal patterns and mating behaviour in wild Bornean orangutans (Pongo pygmaeus). American Journal of Primatology 42: 124 [Abstract].

Knot, C.D. (1998). Changes in Orangutan Caloric Intake, Energy Balance, and Ketones in Response to Fluctuating Fruit Availability. International Journal of Primatology 19: 1061-1079.

Knott, C.D. (2001). Ape models of female reproductive ecology. In Ellison, P.T. (ed.) Reproductive ecology and human evolution, Aldine, Chicago, pp. 429–463.

Koenig, A. (2000) Competitive regimes in forest-dwelling Hanuman langur females (Semnopithecus entellus). Behavioural Ecology and Sociobiology 48:93–109.

Koenig, A., Borries, C., Chalise, M.K. and Winkler, P. (1997). Ecology, nutrition, and timing of reproductive events in an Asian primate, the Hanuman langur (Presbytis entellus). Journal of Zoology 243: 215–235.

Krief, S., Huffman, M.A., Sevenet, T., Guillot, J., Bories, C., Hladik, C.M. and Wrangham, R.W. (2005). Noninvasive monitoring of the health of Pan troglodytes schweinfurthii in the Kibale National Park, Uganda. International Journal of Primatology 26: 467–490.

Krpan, K.M., Coombs, R., Zinga, D., Steiner, M. and Fleming, A.S. (2005). Experiential and hormonal correlates of maternal behavior in teen and adult mothers. Hormones and Behavior 47: 112–122.

Kummer, H. (1971). Primate societies: Group techniques of ecological adaptation. Aldine Atherton, Chicago.

Kunz, B.K. and Linsenmair, K.E. (2008). The Disregarded West: Diet and Behavioural Ecology of Olive Baboons in the Ivory Coast. Folia Primatologica 79: 31-51.

Kurita, H., Suzumura, T., Kanchi, F. and Hamada, Y. (2011). A photogrammetric method to evaluate nutritional status without capture in habituated free-ranging Japanese macaques (Macaca fuscata): a pilot study. Primates 53: 7-11.

LaFleur, M. and Gould, L. (2009). Feeding outside the Forest: The Importance of Crop Raiding and an Invasive Weed in the Diet of Gallery Forest Ring-Tailed Lemurs (Lemur catta) following a Cyclone at the Beza Mahafaly Special Reserve, Madagascar. Folia Primatol 80: 233–246.

Lahm, S.A. (1995). A nationwide survey of crop raiding by elephants and other species in Gabon. Pachyderm 21: 69–77.

Lambert, J.E. (2007). Primate Nutritional Ecology: Feeding Biology and Diet at Ecological and Evolutionary Scales. In Campbell, C.J., Fuentes, A. MacKinnon, K.C. and Panger, M. (eds.), Primates in Perspective, Oxford University Press, Oxford, pp. 482–495.

Landys, M.M., Ramenofsky, M. and Wingfield, J.C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. General Comparative Endocrinology 148: 132–149.

Lasley, B. Hendrickx, A.G. and Stabenfeldt G. H. (1974). Estradiol Levels Near the Time of Ovulation in the Bonnet Monkey (Macaca radiata). Biology of Reproduction 11: 237-244.

Laznic, V., Dincic, E. and Milanovic, I. (2011). The influence of corticosteroid therapy on insulin and c-peptide level in patients with multiple Sclerosis. Clinical Chemistry and Laboratory Medicine 49: S448-S448 [Abstract].

LeBlanc, M., Festa-Bianchet, M. and Jorgenson, J.T. (2001). Sexual size dimorphism in bighorn sheep (Ovis canadensis): effects of population density. Canadian Journal of Zoology 79: 1661–1670.

Lederman, E., Lederman, R. B., Work, B. A. and McCann, D. S. (1981). Maternal psychological and physiologic correlates of fetal-newborn health-status. American Journal of Obstetrics and Gynecology 139: 956-958.

Lee, P.C. (1987). Nutrition, fertility, and maternal investment in primates. Journal of Zoology 213: 409-422.

Lee, P.C. and Hauser, M.D. (1998). Long-term consequences of changes in territory quality on feeding and reproductive strategies of vervet monkeys. Journal of Animal Ecology 67: 347–358.

Leonard, W.R. and Robertson, M.L., (1997). Comparative primate energetics and hominoid evolution. American Journal of Physical Anthropology 102: 265–281.

Linkie, M., Dinata, Y., Nofrianto, A. and Leader-Williams, N. (2007). Patterns and perceptions of wildlife crop raiding in and around Kerinci Seblat National Park, Sumatra. Animal Conservation 10: 127–135.

Lindström, J. (1999). Early development and fitness in birds and mammals. Trends in Ecology and Evolution 14: 343–348.

Liu, Y., Gold, E.B., Lasley, B.L. and Johnson, W.O. (2004). Factors affecting menstrual cycle characteristics. American Journal of Epidemiology 160: 131–140.

Liu, J.H., Burdette, J.E., Xu, H.Y., Gu, C.G., van Breeman, R.B., Bhat, K.P.L., Booth, N., Constantinou, A.I., Pezzuto, J.M., Fong, H.H.S., Farnsworth, N.R., Bolton, J.L. (2001). Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. Journal of Agricultural Food Chemistry 49: 2472–2479.

Lloyd, M.L., Susa, J.B., Pelto, J.A. and Savage, A. (1995) Gestational diabetes mellitus in a white-faced saki (Pithecia pithecia). Journal of Zoo and Wildlife Medicine 26: 76–81.

Lockwood, C.J., Radunovic, N., Nastic, D., Petkovic, S., Aigner, S. and Berkowitz, G.S. (1996). Corticotropin-releasing hormone and related pituitary–adrenal axis hormones in fetal and maternal blood during the second half of pregnancy. Journal of Perinatal Medicine 24: 243–251.

Lu, A., Borries, C., Czekala, N.M., Beehner, J.C. (2009). Natural birth control: seasonal increases in fecal progestins affect reproductive function in wild female Phayre's leaf monkeys (Trachypithecus phayrei). American Journal of Physical Anthropology 138: 178 [Abstract].

Lucas, P.W., Corlett, R.T., Dominy, N.J., Essacklee, H.C., Riba-Hernandez, P., Stoner, K.E. and Yamashita, N. (2003). Dietary analysis II: Food chemistry. In Setchell, J. And Curtis, D.J. (eds.), Field and Laboratory Methods in Primatology, Cambridge University Press, Cambridge, pp.199-213.

Lynch, J.W., Ziegler, T.E. and Strier, K.B. (2002). Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys, Cebus apella nigritus. Hormones and Behavior 41: 275-287.

MacArthur, R.H., Pianka E.R. (1966). The optimal use of a patchy environment. American Naturalist 100: 603-609.

MacLarnon, A.M., Sommer, V., Ellis, J.J., Goffe, A.S., Higham, J.P., Lodge, E., Tkaczynski, P.J., & Ross, C. (2010). Energetics and stress: climatic variation and glucocorticoid levels in forest-living west African baboons. International Society of Primatology XXIII, Kyoto, Japan, September 2010 [Abstract].

Maestripieri, D., Hoffman, C.L., Anderson, G.M., Carter, C.S. and Higley, J.D. (2009). Mother infant interactions in free-ranging rhesus macaques: relationships between physiological and behavioral variables. Physiology and Behavior 96: 613–619.

Malenky, R.K. and Wrangham, R.W. (1994). A quantitative comparison of terrestrial herbaceous food consumption by Pan paniscus in the Lomako Forest, Zaire, and Pan troglodytes in the Kibale Forest, Uganda. American Journal of Primatology 32: 1–12.

Marcus, M.D., Loucks, T.L. and Berga, S.L. (2001). Psychological correlates of functional hypothalamic amenorrhea. Fertility and Sterility 79: 310-316.

Matteo, S. (1987). The effect of job stress and job interdependency on menstrual cycle length, regularity and synchrony. Psychoneuroendocrinology 12: 467-476.

McAdam, A.G. and Millar, J.S. (1999). Dietary protein constraint on age at maturity: an experimental test with wild deer mice. Journal of Animal Ecology 68:733–740.

McGrew, W.C., Tutin, C.E.G., Collins, D.A. and File, S.K. (1989). Intestinal parasites of sympatric Pan troglodytes and Papio spp. at two sites: Gombe (Tanzania) and Mt Assirik (Senegal). American Journal of Primatology 17: 147-155.

McLean, M. and Smith, R., (1999). Corticotropin-releasing hormone in human pregnancy and parturition. Trends in Endocrinology and Metabolism 10: 174–178.

Meistas, M., Rendell, M., Margolis, S. and Kowarski, A., (1982). Estimation of the secretion rate of insulin from urinary excretion rate of C-peptide. Study in obese and diabetic subjects. Diabetes 31: 449–453.

Millspaugh, J.J. and Washburn, B.E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138: 189–199.

Milton, K. (1984). The role of food-processing factors in primate food choice. In Rodman, P.S. and Cant, J.G.H. (eds.) Adaptations for foraging in non-human primates, Columbia University Press, New York, pp. 249-279.

Milton, K. (1996) Effects of bot fly (Alouattamyia baeri) parasitism on a free-ranging howler monkey (Alouatta palliata) population in Panama. Journal of Zoology 239: 39-63.

Miura, K., Nakagawa, H., Nishijo, M., Tabata, M., Morikawa, Y., Ishizaki, M., Yoshita, K. and Kawano, S. (1996). Blood pressure and urinary C-peptide excretion in subjects with varying degrees of glucose tolerance. Blood Pressure 5: 148–153.

Mocanu, V., Vergely, N., Voitellier, P., Rachidi-Kousa, A. and Estour, B. (2003). Correlations between carbohydrate metabolism and corticotrop axis parameters in anorexia nervosa. Pathophysiology 10:37–45.

Mori, A. (1979). Analysis of population changes by measurement of body weight. Primates 20: 371-397.

Mori, A., Yamaguchi, N., Watanabe, K. and Shimizu, K. (1997). Sexual maturation of female Japanese macaques under poor nutritional conditions and food enhanced perianal swelling in the Koshima troop International Journal of Primatology 18: 553-557.

Muehlenbein, M. and Watts, D. (2010). The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. Biopsychosocial Medicine 4: 21.

Muller, M.N. and Lipson, S.F., (2003). Diurnal patterns of urinary steroid excretion in wild chimpanzees. American Journal of Primatology 60: 161–166.

Muller, M.N. and Wrangham, R.W. (2004). Dominance, cortisol and stress in wild chimpanzees (Pan troglodytes schweinfurthii). Behavioural Ecology and Sociobiology 55: 332–340.

Muruthi, P., Altmann, J. and Altmann, S. (1991). Resource base, parity and reproductive condition affect females' feeding time and nutrient intake within and between groups of a baboon population. Oecologia 87: 467–472.

N'guessan, A.K., Ortmann, S. and Boesch, C. (2009). Daily energy balance and protein gain among Pan troglodytes verus in the Taï National Park, Côte d'Ivoire. International Journal of Primatology 30: 481–496.

Nadler, R.D. and Collins, D.C. (1991). Copulatory frequency, urinary pregnanediol, and fertility in great apes. American Journal of Primatology 24: 167–179.

Nakagawa, N. (2000). Seasonal, sex, and interspecific differences in activity time budgets and diets of patas monkeys (Erythrocebus patas) and Tantalus monkeys (Cercopithecus aethiops tantalus), living sympatrically in Northern Cameroon. Primates 41: 161-174.

Narayanan, S. and Appleton, H.D. (1980). Creatinine - a review. Clinical Chemistry 26: 1119–1126.

National Research Council. (2003). Nutrient Requirements of Nonhuman Primates. National Academic Press, Washington D.C.

Nepomnaschy, P.A., Welch, K., McConnell, D., Strassmann, B.I. and England, B.G. (2004). Stress and female reproductive function: a study of daily variations in cortisol, gonadotrophins, and gonadal steroids in a rural mayan population. American Journal of Human Biology 16: 523-532.

Nordlander, E., Hanson, U., Stangenberg, M. and Persson, B. (1989). Twenty-four hour excretion of urinary C-peptide in gestational diabetic women before and after treatment with diet or diet and insulin. Diabetes Research 10: 25-30.

Norris, O. (2007). Vertebrate endocrinology , 4th edition, Elsevier Academic Press, MA, USA.

Nunn, C. L. and Altizer, S. (2006). Infectious diseases in primates: behavior, ecology and evolution, Oxford University Press, Oxford.

Nuttall, F.Q. and Gannon, M.C. (l991). Plasma glucose and insulin response to macronutrients in nondiabetic and NIDDM subjects. Diabetes Care l4: 824-838.

O'Connor, K.A., Brindle, E., Shofer, J., Trumble, B.C., Aranda, J.D., Rice, K. and Tatar, M. (2011). The Effects of a Long-Term Psychosocial Stress on Reproductive Indicators in the Baboon. American Journal of Physical Anthropology 145: 629–638

Oates, J. F. (1978). Water-Plant and Soil Consumption by Guereza Monkeys (Colobus guereza): A Relationship with Minerals and Toxins in the Diet? Biotropica 10: 241-253.

Oates, J.F., Whitesides, G.H., Davies, A.G., Waterman, P., Green, S.M., Dasilva, G.L. and Mole, S. (1990). Determinants of variation in tropical forest primate biomass: new evidence from West Africa. Ecology 71: 328–343.

Oftedal, O.T. (1991). The nutritional consequences of foraging in primates - the relationship of nutrient intakes to nutrient-requirements. Philosophical Transactions of the Royal Society London, Series B 334: 161-170.

Ortiz, R.M., Wade, C.E., Ortiz, C.L. (2001). Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. American Journal of Physiology- Regulatory, Integrative and Comparative Physiology 280: R790–R795.

Packer, C., Collins, D.A., Sindimwo, A. and Goodall, J. (1995). Reproductive constraints on aggressive competition in female baboons. Nature 373: 60–63.

Parr, R.A. (1992). Nutrition–progesterone interactions during early pregnancy in sheep. Reproduction Fertility and Development 4: 297–300.

Parr, R.A., Davis, I.F., Fairclough, R.J. and Miles, M.A. (1987). Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. Journal of Reproduction and Fertility 80: 317–320.

Pereira, R.J.G., Duarte, J.M.B. and Negrao, J.A. (2006). Effects of environmental conditions, human activity, reproduction, antler cycle and grouping on fecal glucocorticoids of free-ranging Pampas deer stags (Ozotoceros bezoarticus bezoarticus). Hormones and Behavior 49: 114–122.

Plotka, E.D., Seal, U.S., Verme, L.J. and Ozoga, J.J. (1983). The adrenal gland in white-tailed deer: a significant source of progesterone. Journal of Wildlife Management 47:38–44.

Polonsky, K.S., Given, B.D., Hirsch, L., Shapiro, E.T., Tillil, H., Beebe, C., Galloway, J.A., Frank, B.H., Karrison, T. and Van Cauter, E. (1988). Quantitative study of insulin secretion and clearance in normal and obese subjects. Journal of Clinical Investigation 81: 435-441.

Post, D.G., Hausfater, G. and McCuskey, S.A. (1980). Feeding behavior of yellow baboons (Papio cynocephalus): relationship to age, gender and dominance rank. Folia Primatologica 34:170–195.

Power, M. (1986). The foraging adaptation of chimpanzees and the recent behaviours of the provisioned apes in Gombe and Mahale National Parks, Tanzania. Human Evolution 1: 251-266.

Pride, R.E. (2005). Foraging success, agonism, and predator alarms: behavioral predictors of cortisol in Lemur catta. International Journal of Primatology 26: 295–319.

Prime, G.R., and Symonds, H.W. (1993). Influence of plane of nutrition on portal blood flow and the metabolic clearance rate of progesterone in ovariectomized gilts. Journal of Agricultural Science 121: 389–397.

Priston, N.C., Wyper, R.W. and Lee, P.C. (2012). Buton Macaques (Macaca ochreata brunnescens): Crops, Conflict, and Behavior on Farms. American Journal of Primatology 74: 29–36.

Pusey, A.E., Oehlert, G.W., Williams, J.M. and Goodall, J. (2005). Influence of Ecological and Social Factors on Body Mass of Wild Chimpanzees International Journal of Primatology 26: 1-31.

R&D Systems (2006). ParameterTM Creatinine Assay, Package Insert, Abingdon, UK.

Raichlen, D.A., Gordon, A.D. and Sechrest, W. (2011) Bioenergetic Constraints on Primate Abundance. International Journal of Primatology 32: 118–133.

Randall. D., Burggren, W. and French, K. (2001). Eckert animal physiology: mechanisms and adaptations. W.H. Freeman & Company, New York, pp. 752.

Rasbash, J., Steele, F., Browne, W. and Prosser, B. (2004). A User's Guide to MLwiN Version 2.0. Institute of Education, London.

Reeder, D.M., Kosteczko, N.S., Kunz, T.H. and Widmaier, E.P. (2004). Changes in baseline and stress-induced glucocorticoid levels during the active period in free-ranging male and female little brown myotis, Myotis lucifugus (Chiroptera: Vespertilionidae). General and Comparative Endocrinology 136: 260–269.

Reeder, D.M., Kramer, K.M., (2005). Stress in free-ranging mammals: integrating physiology, ecology, and natural history. Journal of Mammalogy 86: 225–235.

Robbins, M.M. and Hohmann, G. (2006). Primate feeding ecology: an integrative approach. In Hohman, G., Robbins, M.M. and Boesch, C. (eds.) Feeding Ecology in Apes and Other Primates, Cambridge University Press, Cambridge, pp. 1-13.

Robinson, A.M. and Williamson, D.H. (1980). Physiological Roles of Ketone Bodies as Substrates and Signals in Mammalian Tissues. Physiological Review. 60: 143-187.

Rolland, R. M., Hausfater, G., Marshall, B. and Levy, S. (1985). Antibiotic-resistant bacteria in wild primates: Increase prevalence in baboons feeding on human refuse. Applied Environmental Microbiology 49: 791–794.

Romero, L.M., (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General Comparative Endocrinology 128: 1–24.

Romero, L.M., (2004). Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19: 249–255.

Rose, L. (1994) Sex differences in diet and foraging behavior in white-faced capuchins (Cebus capuchinus) International Journal of Primatology 15: 95-114.

Ross, C., Warren, Y., MacLarnon, A.M. and Higham, J.P. (2011) How Different Are Gashaka's Baboons? Forest and Open Country Populations Compared. In Sommer, V. and Ross, C. (eds.), Primates of Gashaka, Springer, New York, NY, pp. 333-360.

Rothman, J.M., Dierenfeld, E.S. Hintz, H.F. and Pell, A.N. (2008). Nutritional quality of gorilla diets: consequences of age, sex, and season. Oecologia 155: 111–122.

Rybkin, I.I., Zhou, Y., Volaufova, J., Smagin, G.N., Ryan, D.H. and Harris, R.B. (1997). Effect of restraint stress on food intake and body weight is determined by time of day. American Journal of Physiology 273: 1612–22.

Salimetrics. (2010). High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit. Salimetrics Europe, Ltd, UK.

Saltz, D. and White, G.C. (1991). Urinary cortisol and urea nitrogen responses to winter stress in mule deer. Journal of Wildlife Management 55: 1-16.

Saltzman, W. and Abbott, D.H. (2009). Effects of Elevated Circulating Cortisol Concentrations on Maternal Behavior in Common Marmoset Monkeys (Callithrix jacchus). Psychoneuroendocrinology 34: 1222–1234.

Sangsritavong, S., Combs, D.K., Sartori, R., Amentano, L.E. and Wiltbank, M.C. (2002). High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. Journal of Dairy Science 85: 2831–2842.

Sapolsky, R.M. (1986). Endocrine and behavioral correlates of drought in the wild baboon. American Journal of Primatology 11: 217-228.

Sapolsky, R.M. (1992). Cortisol concentrations and the social significance of rank instability among wild baboons. Psychoneuroendocrinology 17: 701-709.

Sapolsky, R.M., Romero, L.M. and Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrinological Review 21: 55–89.

Schmidt-Nielsen, K. (1997). Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge.

Schneider, J.E. and Wade, G.N. (2000). Inhibition of reproduction in service of energy balance. In Wallen, K. and Schneider, J.E. (eds.) Reproduction in context: social and environmental influences on reproduction. MIT Press, Cambridge, pp. 35–82.

Schoech, S.J., Bowman, R., Bridge, E.S. and Boughton, R.K. (2007). Baseline and acute levels of corticosterone in Florida scrubjays (Aphelocoma coerulescens): effects of food supplementation, suburban habitat, and year. General Comparative Endocrinology 154: 150–160.

Schuur, E.A.G. (2003). Productivity and global climate revisited: the sensitivity of tropical forest growth to precipitation. Ecology 84: 1165–1170.

Semple, S., Cowlishaw, G. and Bennett, P. (2002). Immune system evolution among anthropoid primates: parasites, injuries and predators. Proceedings of the Royal Society of London, Series B 269: 1031–1037.

Setchell, J.M., Smith, T., Wickings, E.J. and Knapp, L.A. (2008). Factors affecting fecal glucocorticoid levels in semi-free-ranging female mandrills (Mandrillus sphinx). American Journal of Primatology 70: 1023–1032.

Sherry, D.S. and Ellison, P.T. (2007). Potential Applications of Urinary C-Peptide of Insulin for Comparitive Energetics Research. American Journal of Physical Anthropology 133: 771-778.

Shutt, K., MacLarnon, A., Heistermann, M. and Semple, S. (2007). Grooming in Barbary macaques: Better to give than receive? Biological Letters 3: 231–233.

Siex, K.S., and Struhsaker, T.T. (1999). Colobus monkeys and coconuts: a study of perceived human-wildlife conflicts. Journal of Applied Ecology 36: 1009-1020.

Silk, J.B. (1987). Activities and Feeding Behavior of Free-Ranging Pregnant Baboons. International Journal of Primatology 8: 593-613.

Silk, J.B., Willoughby, E. and Brown, G.B (2005). Maternal rank and local resource competition do not predict birth sex ratios in wild baboons Proceedings of the Royal Society, Series B 272: 859-864.

Singh, M. and Vinathe, S. (1990). Inter-population differences in the time budgets of bonnets monkeys (Macaca radiata). Primates 31: 589-596.

Sliutz, G., Spieser, P., Schultz, A., Spona, J. and Zeillinger, R. (1993). Agnus castus extracts inhibit prolactin secretion of rat pituitary cells. Hormone and Metabolic Research 25: 253–255.

Small, M. 1981. Body fat, rank, and nutritional status in a captive group of rhesus macaques. International Journal of Primatology 2: 91–95.

Smith, R.J. and Jungers, W.L. (1997). Body mass in comparative primatology. Journal Human of Evolution 32: 523–559.

Smith, T.F. and French, J.A. (1997). Social and reproductive conditions modulate urinary cortisol excretion in black tufted-ear marmosets (Callithrix kuhli). American Journal of Primatology 42: 253– 267.

Sommer, V., Adanu, J., Faucher, I. and Fowler, A. (2004). Nigerian chimpanzees (Pan troglodytes vellerosus) at Gashaka: two years of habituation efforts. Folia Primatologica 75: 295–316.

Sommer, V., Bauer, J., Fowler, A. and Ortmann, S. (2011). Patriarchal Chimpanzees, Matriarchal Bonobos: Potential Ecological Causes of a Pan Dichotomy. In Sommer, V. and Ross, C. (eds.), Primates of Gashaka, Springer, New York, NY, pp. 417-449.

Sommer, V. and Ross, C. (2011). Exploring and Protecting West Africa's Primates: The Gashaka Primate Project in Context. In Sommer, V. and Ross, C. (eds.), Primates of Gashaka, Springer, New York, NY, pp. 1-38.

Sousa, M.B.C. and Ziegler, T.E. (1998). Diurnal variation on the excretion patterns of fecal steroids in common marmoset (Callithrix jacchus) females. American Journal of Primatology 46: 105–117.

Srivastava, A. (1992). Uses of resources by free-ranging female langurs (Presybtis entellus) during different reproductive phases. Folia Primatologica 59: 157-162.

Stacey, P.B. (1986). Group size and foraging efficiency in yellow baboons. Behavioural Ecology and Sociobiology 18: 175–187.

Stelzner, J.K. (1988) Thermal effects on movement patterns of yellow baboons. Primates 29: 91–105.

Sterck, E.H.M., Watts, D.P. and van Schaik, C.P. (1997). The evolution of female social relationships in nonhuman primates. Behavioural Ecology and Sociobiology 41: 291-309.

Stacey, P.B. (1986). Group size and foraging efficiency in yellow baboons. Behavioural Ecology and Sociobiology 18: 175 187.

Stavisky, R.C., Whitten, P.L., Hammett, D.H. and Kaplan, J.R. (1997). The use of Lake pigments to examine the relationship between dominance and fecal cortisol levels in group-housed Macaca fascicularis. American Journal of Physical Anthropology 104: 217-218.

Strier, K.B. (2003). Primate Behavioral Ecology, 2nd Edition. Allyn and Bacon, London.

Strum, S. (1991). Weight and age in olive baboons. American Journal of Primatology 25: 219-237.

Strum SC. (1994). Prospects for management of primate pests. Revue d'Ecologie (la Terre et la Vie) 49: 295-306.

Strum, S.C. (2010). The Development of Primate Raiding: Implications for Management and Conservation. International Journal of Primatology 31: 133–156.

Sugiyama, Y. and Oshawa, H. (1982). Population dynamics of Japanese monkeys with special reference to artificial feeding. Folia Primatologica 39: 238-263.

Suzuki, Y., Oka, Y., Taniyama, M., Murata, C., Atsumi, Y. and Matsuoka, K. (2004). A case of type 2 diabetes with high levels of plasma and urinary C-peptide. Diabetes Research and Clinical Practice 66: S125-S128.

Tamashiro, K.L., Hegeman, M.A., Nguyen, M.M., Melhorn, S.J., Ma, L.Y., Woods SC, and Sakai, R.R. (2007). Dynamic body weight and body composition changes in response to subordination stress. Physiology and Behavior 91: 440–448.

Tamashiro, K.L., Hegeman, M.A. and Sakai, R.R. (2006). Chronic social stress in a changing dietary environment. Physiology and Behavior 89: 536–42.

Taussky, H.H. (1954). A microcolorimetric determination of creatinine in urine by the Jaffe reaction. Journal of Biological Chemistry 208: 853–861.

Taylor, C.R., Schmidt-Nielsen, K. and Raab, J.L. (1970). Scaling of energetic cost of running to body size in mammals. American Journal of Physiology 219: 1104–1107.

Tharp, G.D. (1975). The role of glucocorticoids in exercise. Medicine and Science in Sports 7: 6–11.

Toledo, M., Poorter, L., Peñ a-Claros, M., Alarcón, A., Balcázar, J., Leaño, C., Licona, J.C., Llanque, O., Vroomans, V., Zuidema, P. and Bongers, F. (2011) Climate is a stronger driver of tree and forest growth rates than soil and disturbance. Journal of Ecology 99: 254–264

Trivers, R.L. (1972). Parental investment and sexual selection. In Campbell, B. (ed.), Sexual selection and the descent of man 1871-1971, Aldine, Chicago, pp. 136–179.

Valeggia, C. and Ellison, P.T. (2009). Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. American Journal of Human Biology 21: 559–566.

van Noordwijk, M.A. and van Schaik, C.P. (1999). The effects of dominance rank and group size on female lifetime reproductive success in wild long-tailed macaques, Macaca fascicularis. Primates 40: 105–130.

van Schaik, C.P., van Noordwijk, M.A., van Bragt, T. and Blankenstein, M.A. (1991). A pilot study of the social correlates of levels of urinary cortisol, prolactin, and testosterone in wild long-tailed macaques (Macaca fascicularis). Primates 32: 345–356.

van Soest, P.J. (1982). The Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca.

van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Diary Science 74: 3583–3597.

Verme, L.J. (1965). Reproduction studies on penned white-tailed deer. Journal of Wildlife Management 29: 74-79.

Vervaecke, H., de Vries, H. and van Elsacker L. (2000). Dominance and its behavioral measures in a captive group of bonobos (Pan paniscus). International Journal of Primatology 2: 47–68.

Vitzthum, V.J., Spielvogel, H. and Thornburg, J. (2004). Interpopulational differences in progesterone levels during conception and implantation in humans. Proceedings of the National Academy of Sciences, U.S.A. 101: 1443–1448

Vitzthum, V.J. (2009). The Ecology and Evolutionary Endocrinology of Reproduction in the Human Female. Yearbook of Physical Anthropology 52: 95–136.

Vogel, E. R. (2005). Rank differences in energy intake rates in white-faced capuchin monkeys, Cebus capucinus: The effects of contest competition. Behavioural Ecology and Sociobiology 58: 333–344.

Wagner, J.D., Kavanaugh, K., Ward, G.M., Auerbach, B.J., Harwood, H.J. Jr and Kaplan, J.R. (2006). Old World primate models of type 2 diabetes mellitus. ILAR Journal 47: 259-271.

Wahren, J., Ekberg, K. and Jornvall, H. (2007). C-peptide is a bioactive peptide. Diabetologia 50: 503–509.

Wallner, B., Möstl, E., Dittami, J. and Prossinger, H. (1999). Fecal glucocorticoids document stress in female Barbary macaques (Macaca sylvanus). General Comparative Endocrinology 113: 80–86.

Warren, Y., Buba, B. and Ross, C. (2007). Patterns of crop-raiding by wild and domestic animals near Gashaka Gumti National Park Nigeria. International Journal of Pest Management 53: 207–216.

Warren, Y. (2003). Olive Baboons (Papio cynocephalus anubis) Behaviour, Ecology and Human Conflict in Gashaka Gumti National Park, Nigeria. PhD thesis, Roehampton, University of Surrey.

Warren, Y., Higham, J.P., MacLarnon, A.M. and Ross, C. (2011) Crop-raiding and Benefits of Living with Humans. In Sommer, V. and Ross, C. (eds.), Primates of Gashaka, Springer, New York, NY, pp. 307-332.

Wasser, S.K., Davenport, B., Ramage, E.R., Hunt, K.E., Parker, M., Clarke, C. and Stenhouse, G. (2004). Scat detection dogs in wildlife research and management: application to grizzly and black bears in the yellowhead ecosystem, Alberta, Canada. Canadian Journal of Zoology 82: 475–492.

Wasser, S.K., (1996). Reproductive control in wild baboons measured by fecal steroids. Biology of Reproduction 55: 393–399.

Wasserman, M.D. and Chapman, C.A. (2003). Determinants of colobine monkey abundance: the importance of food energy, protein and fibre content. Journal of Animal Ecology 72: 650–659.

Waterman, P.G., Ross, J.A.M., Bennett, E.L. and Davies, A.G. (1988). A comparison of the floristics and leaf chemistry of the tree flora in two Malaysian rain forest and the influence of leaf chemistry on populations of colobine monkeys in the Old World. Biological Journal of the Linnean Society 34: 1–32.

Watts, D. P. (1998). Seasonality in the ecology and life histories of mountain gorillas (Gorilla gorilla beringei). International Journal of Primatology19: 929-948.

Weingrill, T., Gray, D.A., Barrett, L. and Henzi, S.P. (2004). Fecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. Hormones and Behavior 25: 259-269.

Weiss, R.F., (1988). Herbal Medicine. Ab arcanum, Sweden.

Weyher, A.H., Ross, C. and Semple, S. (2006). Gastrointestinal Parasites in Crop Raiding and Wild Foraging Papio anaubis in Nigeria. International Journal of Primatology 27: 1519-1534.

Whiten, A., Byrne, R., Barton, R.A., Waterman, P.G. and Henzi, S.P. (1991). Dietary and foraging strategies of baboons. Philosophical Transactions of the Royal Society, Series B 334: 187-197.

Whitten, P. L. (1983). Diet and dominance among female vervet monkeys (Cercopithecus aethiops). American Journal of Primatology 5: 139–159.

Whitten, P.L., Brockman, D.K., and Stavisky, R.C. (1998). Recent advances in noninvasive techniques to monitor hormone–behaviour interactions. American Journal of Physical Anthropology 27: 1–23.

Williams, A.H., and Cumming, I.A. (1982). Inverse relationship between concentration of progesterone and nutrition in ewes. Journal of Agricultural Science 98: 517–522.

Williams, N.I., Berga, S.L. and Cameron, J.L. (2007). Synergism between psychosocial and metabolic stressors: impact on reproductive function in cynomolgus monkeys. American Journal of Physiology, Endocrinology and Metabolism 293: E270–E276.

Wingfield, J.C. and Sapolsky, R.M. (2003). Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15: 711–724.

Wolden-Hanson, T., Davis, G.A., Baum, S.T. and Kemnitz, J.W. (1993). Insulin levels, physical activity, and urinary catecholamine excretion of obese and non-obese rhesus monkeys. Obesity Research 1: 5–17.

Wrangham, R.W. (1974). Artificial feeding of chimpanzees and baboons in their natural habitat. Animal Behaviour 22: 83-93.

Wrangham, R.W. (1980). An ecological model of female-bonded primate groups. Behaviour 75:262–300

Wu Leung, W.T. (1968). Food Composition Table for Use in Africa. Bethesda, Department of Health, Education and Welfare, MD, U.SA.

Xiao, E., Xia-Zhang, L. and Ferin, M. (1999). Stress and the menstrual cycle: short- and long-term response to a five-day endotoxin challenge during the luteal phase in the rhesus monkey. Journal of Clinical Endocrinology and Metabolism 84: 623–626.

Xiao, E., Xia-Zhang, L. and Ferin, M. (2002). Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the rhesus monkey. Journal of Clinical Endocrinology and Metabolism 87: 2232-2237.

Xiao, E., Xia-Zhang, L., Shanen, D. and Ferin, M. (1997). Tonic support of luteinizing hormone secretion by adrenal progesterone in the ovariectomized monkey replaced with midfollicular phase levels of estradiol. Journal of Clinical Endocrinology and Metabolism 82: 2233–2238.

Yoshida, N.M., Yoshiuchi, K., Kumano, H., Sasaki, T. and Kuboki, T. (2006). Changes in heart rate with refeeding in anorexia nervosa: a pilot study. Journal of Psychosomatic Research 61: 571-575.

Zakar, T. and Mitchell, B. (1998). The endocrinology of late pregnancy and parturition. In Bittar, E.E. and Bittar, N (eds.), Reproductive endocrinology and biology, JAI Press, Greenwich, pp. 167–182.

Ziegler, T., Hodges, K., Winkler, P. and Heistermann, M. (2000). Hormonal correlates of reproductive seasonality in wild female Hanuman langurs (Presbytis entellus). American Journal of Primatology 51: 119–134.

Ziegler, T.E., Scheffler, G. and Snowdon, C.T. (1995). The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, Saguinus oedipus. Hormones and Behavior 29: 407– 424.

Zinner, D., Buba, U., Nash, S. and Roos, C. (2011). Pan-African Voyages: The Phylogeography of Baboons. In Sommer, V. and Ross, C. (eds.), Primates of Gashaka, Springer, New York, NY, pp. 307-332.